EGG FOOD SAFETY SCHEME

PERIODIC REVIEW OF THE RISK ASSESSMENT

NSW GOVERNMENT Food Authority

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Glossary

ABS	Australian Bureau of Statistics
AI	Avian Influenza
AMR	antimicrobial resistance
APVMA	Australian Pesticides and Veterinary Medicines Authority
ASTAG	Australian Strategic and Technical Advisory Group on AMR
ATDS	Australian Total Diet Study
AVPA	Australasian Veterinary Poultry Association
BEIC	British Egg Industry Council
CAC	Codex Alimentarius Commission
CDC	The Centers for Disease Control and Prevention (USA)
CFR	Code of Federal Regulations
CLSI	Clinical and Laboratory Standards Institute
CIA	critically important antibiotic
CSIRO	Commonwealth Scientific and Industrial Research Organisation (Australia)
CTEs	Critical Tracking Events
DAE	days after exposure
DAFF	Department of Agriculture, Fisheries and Forestry (Australia)
DAWE	Department of Agriculture, Water and the Environment (Australia)
DAWR	Department of Agriculture and Water Resources (Australia)
DCPs	Dangerous Contact Premises
DOA	dead on arrival
DoH	Department of Health (Australia)
ECOFFs	epidemiologic cut-off values
ECS	Emergency Control Scheme
EFSA	European Food Safety Authority
ELISA	enzyme linked immunosorbent assay
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organisation
FDA	Food and Drug Administration (USA)
FRSC	Food Regulation Standing Committee (Australia)
FSA	Food Standards Agency (UK)
FSAI	Food Safety Authority of Ireland
FSANZ	Food Standards Australia New Zealand
FSIS	Food Safety and Inspection Service (USA)
FSS	Food Safety Supervisor
FTL	Food Traceability List
GMPs	Good Management Practices
HACCP	Hazard Analysis and Critical Control Point
HPAI	high-pathogenic Avian Influenza
IBD	Individual Biosecurity Direction
IP	Infected Property
KDEs	Key Data Elements
LEDS	Laboratory-based Enteric Disease Surveillance (LEDS) system (USA)
MCDA	multiple cross-displacement amplification (MCDA) assay
MCR	multi-class resistant
MIC	minimum inhibitory concentration
MPI	Ministry for Primary Industries (NZ)
NAHMS	National Animal Health Monitoring System (USA)
NRS	National Residue Survey
NSEMAP	National *Salmonella* Enteritidis Monitoring & Accreditation Program
NSW	New South Wales
NSW DPI	New South Wales Department of Primary Industries
NSW LLS	New South Wales Local Land Services
NZ	New Zealand
OIE	World Organisation for Animal Health
PCR	polymerase chain reaction
PIC	Property Identification Code

PT	phage type
RDNC	Reaction Does Not Conform
RFID	radio frequency identification
SA	South Australia
SE	*Salmonella enterica* serovar Enteritidis
SE 7A	Australian isolate *Salmonella* Enteritidis 7A
SFPQ	Safe Food Production Queensland
SH	*Salmonella enterica* serovar Hadar
SI	*Salmonella enterica* serovar Infantis
SOPs	Standard Operating Procedures
ST	*Salmonella enterica* serovar Typhimurium
SV	*Salmonella enterica* serovar Virchow
TAS	Tasmania
TPs	Trace Premises
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
VIC	Victoria
QLD	Queensland
WA	Western Australia
WGS	whole genome sequencing
WHO	World Health Organisation
YMT	Yolk Mean Time

Units of measurement

°C	degrees Celsius
°F	degrees Fahrenheit
cfu	colony forming units
cm	centimetre
fg	femtogram
g	gram
mt	metric tonne
s	second

Executive summary

The previous risk assessment of the egg food safety scheme was published in June 2013 (NSW Food Authority, 2013b). The 2013 risk assessment was an update of the 2009 risk assessment. Each five-year review is conducted on an alternate basis, as either a full risk assessment or an update. A full risk assessment is reported here containing new or updated information identified in an environmental scan for issues related to eggs that have impacted egg product food safety since 2013.

Information sources included;

- foodborne illness reports and recall data in Australia attributed to eggs and egg products
- international issues arising from human illness or perceived hazards linked with eggs and egg products
- risk assessments of eggs and egg products
- emerging issues in the farm to consumer continuum for eggs and egg products relevant to health risk
- research findings related to hazards in eggs and egg product production and processing
- baseline surveys of microbiological and chemical hazards in eggs and egg products
- other relevant sources if identified during the above activities

In the 2013 risk assessment *Salmonella* Enteritidis (SE) was not specifically considered a hazard, as most human cases of SE in Australia at the time arose from travellers returning from overseas. SE presents a higher risk to consumers and the Australian egg industry because it can colonise the internal contents of eggs during their development via infection of the chicken oviduct. As well as being reported to be more successful than other *Salmonella* serotypes in colonising the chicken oviduct, SE has been proposed to possess an enhanced ability to survive in the hostile egg white compartment.

The hazard identification and main findings of the 2013 risk assessment have been updated in line with the first reported locally acquired SE egg-related outbreak and the first detection of SE in New South Wales (NSW) poultry in September 2018. A total of 17 premises were recorded as an Infected Premise (IP) in NSW following the start of the SE outbreak. The 17 IPs were located in Colo Vale, Greater Sydney, Mangrove Mountain and Port Stephens. The 17 IPs were found to have links to each other through a complex network of movement of people, vehicles and equipment. The number of other poultry properties (including other IPs) that each of the 17 IPs had direct links to, ranged from just one other poultry property to up to 15. Compounding the complexity of the outbreak investigation was the identification of an IP with unregistered flocks and the movement of unstamped/ungraded eggs between multiple IPs. In mid-2019, NSW DPI introduced the Biosecurity (*Salmonella* Enteritidis) Control Order 2019 to assist in the management of the biosecurity risk posed by the spread of SE. The order established minimum biosecurity standards for the poultry and egg industries and made them legally enforceable under the *Biosecurity Act 2015* (NSW). Under a revised control order introduced in mid-2020, licensed egg producers are also required to regularly test for SE. The Control Order in NSW is in effect until the 30th of June 2024. The Biosecurity & Food Safety Compliance team was tasked to inspect all licensed egg farms in NSW to ensure compliance with the Control Order. Areas pertaining to the *Biosecurity Act 2015* and *Food Act 2003* requirements, where a low compliance level was observed included; signage defining the production area, rodent control, record keeping with respect to deliveries and entry to premises and production areas, appropriate fixtures/fittings/equipment in processing areas and, hygiene/sanitation/cleanliness of premises and equipment. As of the 1st of July 2021, all licensed egg farms in NSW were considered compliant with the Control Order. At the time of writing, NSW is still in management mode and undertaking SE decontamination and clearance activities at IPs.

Data supporting the exposure assessment has been updated with the addition of a summary of the consumption data reported in the 25th Australian Total Diet Study (ATDS) (FSANZ, 2019a), the Australian Bureau of Statistics (ABS) (ABS, 2020a) and Australian Eggs Limited (Australian Eggs Limited, 2021a). While each of the sources employed different methodologies to determine the consumption values, overall, egg consumption was reported to have increased. Data released by Australian Eggs Limited shows that average consumption in Australia has risen to 247 eggs per person per year (Australian Eggs Limited, 2021a).

The following overview summarises the update of the hazard characterisation, in relation to foodborne illness in NSW from 2013 to 2020 due to eggs and egg products (Communicable Diseases Branch, 2014, 2015, 2016, 2017, 2018, 2019, *In print-a*, *In print-b*):

- Eggs; alone or in a complex food, were identified as the suspected or responsible vehicle in a total of 52 outbreaks from 2013 to 2020. Where the suspected or responsible vehicle was reported and involved a complex food, multiple outbreaks were linked to raw egg mayonnaise (10%; 5/52), other raw egg sauces *e.g. aioli, béarnaise* (10%; 5/52), Vietnamese rolls (10%; 5/52), tiramisu (8%; 4/52), fried ice-cream (6%; 3/52) and raw egg salad dressings *e.g. caesar* (4%; 2/52).
- The suspected or responsible vehicle was described as "*raw*" or "*undercooked*" in 48% (25/52) and 17% (9/52) of cases, respectively. Cross contamination was implicated in 15% (8/52) of cases.
- *Salmonella* was the causative agent of all (100%; 52/52) egg-related outbreaks. The most common serovar implicated was *S*. Typhimurium (83%; 43/52), followed by *S*. Enteritidis (14%; 7/52) and *S*. Virchow (2%; 1/52). In one of the outbreaks (2%; 1/52), the serotype was not determined.
- Where a setting was associated with multiple egg-related outbreaks, restaurants were implicated in the majority of outbreaks (50%; 26/52), followed by bakeries (15%; 8/52), private residences (14%; 7/52) and take-away venues (12%; 6/52).

The following provides a brief summary of the update of the hazard characterisation, in relation to various recent domestic reports investigating the prevalence and serotypes of *Salmonella* on egg farms and egg grading facilities:

- The NSW Food Authority surveillance program for egg farms and egg grading facilities has reported that the majority of egg businesses surveyed from July 2013 to June 2018 (62%; 33/53) were positive for *Salmonella*. This is a higher prevalence than previously reported in an egg farm survey conducted by the NSW Food Authority between December 2010 and November 2011 (45%; 22/49). A total of 21 serovars were identified, with *S*. Bareilly (2013 - 2014 and 2015 - 2016) and *S*. Infantis (2014 - 2015, 2016 - 2017 and 2017 - 2018) the most common serotypes detected. *S*. Typhimurium was the second most common serotype isolated, either alone or alongside other serotypes (2013 - 2014, 2014 - 2015 and 2016 – 2017).
- In Queensland (QLD), two microbiological surveys of egg farms were undertaken in 2014 (Cuttell, Groves, & Wilson, 2014) and 2015 (Safe Food Production Queensland, 2015). Both surveys included different production types (cage, barn and free-range) and production volumes (small, medium and large). The percentage of QLD egg farms surveyed on which *Salmonella* was detected was 57% (12/21) in 2014 and 55% (15/27) in 2015. *S*. Typhimurium was the most common serovar when the results were examined at the farm-level in 2014 (14%; 3/21) and 2015 (33%; 9/27).
- In Western Australia (WA), a microbiological survey was conducted on commercial layer farms between November 2017 and June 2018 (Sodagari et al., 2020). *Salmonella* was isolated from 100% (7/7) of all egg businesses surveyed and from 88.5% (23/26) of the sampled flocks. *S*. Typhimurium (64.5%; 60/93) and *S*. Infantis (22.5%; 21/93) were the most prevalent serovars.

- National recalls:
  - Between the 17/10/2015 and 31/12/2020, there was a total of nine consumer level recalls of egg and egg products. This included seven recalls due to microbial contamination and two recalls involving inadequate egg

cleaning and the potential for unacceptable dirty eggs. All seven recalls involving microbial contamination were a result of the detection of SE, with 6 consumer level recalls of eggs from implicated properties in NSW and one consumer level recall in Victoria.

While the risk characterisation has largely focused on the control of SE, many recommended SE risk reduction practices are also applicable against other salmonellae as well as a broad spectrum of other poultry pathogens. *S*. Typhimurium (ST) was responsible for 83% of all egg-related outbreaks from 2013 to 2020. However, the number of cases of foodborne salmonellosis attributed to ST declined by 65% from 2014 to 2018 and this has been attributed to efforts in line with the NSW Food Safety Strategy 2015–2021 and the largescale vaccination of layer flocks against ST. The vast majority of egg-related outbreaks were due to ingestion of "*raw*" or "*undercooked*" eggs or egg products. As restaurants, bakeries, private residences and take-away venues were most commonly implicated in these outbreaks, further promotion of educational material already prepared by the NSW Food Authority is warranted. Guidance materials prepared for retail and food service businesses and consumers include documents on risky egg handling practices (NSW Food Authority, 2020e), safe egg handling practices (NSW Food Authority, 2021c) and the safe preparation of raw egg products (NSW Food Authority, 2016a). The *Food Act 2003* (NSW) requires certain food businesses in the NSW hospitality and retail food service sector to have at least one trained Food Safety Supervisor (FSS) (NSW Food Authority, 2022a). As of the 1st of September 2015, to be certified as an FSS for a food premise in NSW and issued a valid certificate, a person must attain required units of competency including those in safe egg handling (NSW Food Authority, 2022b).

The spread of SE in Australia is considered a significant biosecurity risk. In countries in which SE is endemic, comprehensive control programs incorporating a combination of broad-spectrum risk reduction practices plus vaccination and testing targeted to SE, have achieved documented success in reducing both the prevalence of infection in egg-laying flocks and the incidence of disease transmission to humans. Only partial protection from the isolated Australian SE strain was reported to be offered by one of the ST vaccines currently approved for use in laying flocks in Australia (Clark et al., 2021).

At the time of writing this Risk Assessment, Food Standards Australia New Zealand (FSANZ) was concurrently conducting a review of Standard 4.2.5 - *Primary Production and Processing Standard for Eggs and Egg Products*. FSANZ identified a range of control measures for consideration as part of a national approach to manage SE, including biosecurity measures, flock testing, temperature control for intact shell eggs and traceability. Risk reduction practices for SE are inter-dependent, as currently there are no control measures which ensure full protection against SE. Continued collection of information on SE prevalence in the Australian egg-laying environment will be vital to provide a baseline for assessing the effectiveness and appropriateness of any implemented SE risk reduction strategies.

As SE can be highly persistent in both infected birds and diverse environmental reservoirs, complete eradication of SE from the Australian egg production environment is challenging. It is prudent to view SE as part of the broad ecosystem and therefore a continuing threat to commercial egg production. Continued vigilance and maintenance of rigorous biosecurity practices within and between premises and strict hygiene procedures will be crucial for the Australian egg industry into the future.

1. Introduction

The definition of an egg within the Australia New Zealand Food Standards Code (the Code), is an egg from any avian (bird) species, except ratites. The Australian egg industry is largely based on eggs and egg products produced from chickens (*Gallus gallus domesticus*). Other egg-producing avian species, such as ducks, quails, geese, pigeons and guinea fowl form a minor part of the egg market. Although the focus of the assessment is on eggs from chickens, it is assumed that the hazards of concern for other poultry species are largely the same. Unless specifically stated, further references in this report are to chicken eggs.

Egg producers need to meet the requirements of the Food Regulation 2015 and the Food Standards Code.

1.1 NSW Food Regulation 2015

The Food Regulation 2015 underpins the NSW Food Authority's food regulatory work, which aims to reduce the incidence of foodborne illness linked to certain food sectors in NSW [for a review see (NSW Food Authority, 2020d)]. It is important to the food industry as it sets minimum food safety requirements for food industry sectors that have been identified as higher risk, including the egg industry.

These businesses are subject to Food Safety Schemes because of the priority classification. Under each scheme there are licence categories that specify the types of activities each business is licensed to perform.

Businesses within the egg industry that need to hold a licence with the Food Authority include producers (other than small egg farms), graders, processors and storage facilities. While small egg farms do not need to apply for a NSW Food Authority licence, they must 'notify' the Food Authority with their business details and food activities and they must meet any requirements relevant to their business (NSW Food Authority, 2021d).

Egg food businesses need to meet food safety and labelling requirements which vary depending on business type and size:

- small egg farms (producing fewer than 20 dozen (240) eggs/week)
- egg producers (producing more than 20 dozen (240) eggs/week)
- egg graders
- egg processors (businesses that manufacture and/or pasteurise egg products)
- egg storage facilities (other than storing whole eggs)
- egg transporters (other than transporting whole eggs)

The NSW Food Authority has prepared the NSW Food Safety Schemes Manual to specify testing requirements for the Food Safety Schemes under the Food Regulation 2015 (NSW Food Authority, 2020c). The requirements referred to in the Manual are mandatory. The Egg Food Safety Scheme details requirements for sampling and analysis for egg businesses around the microbiological testing of non-reticulated water, egg products and blended egg product mixtures (a product with at least 80% by weight of egg white or yolk or both).

1.2 Egg and egg product facilities in NSW

Poultry are commercially farmed for egg production in every state and territory in Australia except the Northern Territory (Australian Eggs Limited, 2021a). The NSW egg industry is responsible for 34% of all egg production in Australia (NSW Food Authority, 2021b). The Food Authority licenses approximately 300 businesses in this industry.

1.3 Legislation, Standards and Industry Guidelines applicable to egg businesses

The Australia and New Zealand (NZ) food regulatory system involves the Australian Government, NZ and Australian states and territories. In this system food standards are developed under the Code (FSANZ, 2019d), which is administered by Food Standards Australia New Zealand (FSANZ) and enforced by state and territory governments.

The standards in the Code are legislative instruments under the *Legislation Act 2003*. The NSW Food Authority enforces the *Food Act 2003* (NSW) and associated regulations within NSW in respect of all food for sale.

Depending on the type of egg business (NSW Food Authority, 2021b), the following requirements set out in Food Standards Code may apply:

Small egg farms –

- relevant sections of Chapter 1 - General Food Standards
- Standard 2.2.2 - Egg and Egg Products
- Standard 3.2.2 - Food Safety Practices and General Requirements
- Standard 3.2.3 - Food Premises and Equipment

Egg producers -

- Standard 2.2.2 - Egg & Egg Products
- Standard 3.2.1 - Food Safety Programs
- Standard 3.2.2 - Food Safety Practices & General Requirements
- Standard 3.2.3 - Food Premises and Equipment
- Standard 4.2.5 - Primary Production and Processing Standard for Eggs and Egg Products

The Food Authority recommends that egg producers should[1] also implement practices around the receival of sourced birds, stock food, biosecurity, environmental surveillance, drinking water, egg collection, storage of eggs and the cleaning of premises and equipment (NSW Food Authority, 2015e).

Egg graders -

- Standard 2.2.2 - Egg & Egg Products
- Standard 3.2.1 - Food Safety Programs
- Standard 3.2.2 - Food Safety Practices & General Requirements
- Standard 3.2.3 - Food Premises and Equipment

The Food Authority recommends that egg graders should also implement practices around the storage of eggs (NSW Food Authority, 2015d).

Egg processing businesses -

- Chapter 1, Part 1.2 – Labelling and Other Information Requirements
- Standard 1.2.2 – Food Identification Requirements
- Standard 1.2.3 – Mandatory Warnings and Advisory Statements and Declarations
- Standard 1.6.2 – Processing Requirements
- Standard 2.2.2 – Egg & egg products
- Standard 3.2.1 Food Safety Programs Standard
- Standard 3.2.2 Food Safety Practices and General Requirements
- Standard 3.2.3 Food Premises and Equipment
- Standard 4.2.5 - Primary production and processing Standard for Eggs and Egg Product

Egg storage businesses and Egg transport facilities -

- Chapter 1, Part 1.2 - Labelling and Other Information Requirements
- Standard 3.2.2 - Food Safety Practices & General Requirements

[1] The use of the word ‘should’ means that these practices are recommended but not legally required.

- Standard 3.2.3 - Food Premises and Equipment

The Food Authority recommends that egg storage businesses and egg transport businesses should service all refrigeration units annually to ensure efficient operation (NSW Food Authority, 2016b, 2016c). The Food Authority also recommends that transport vehicles should be pre-cooled before transportation occurs (NSW Food Authority, 2016c).

1.3.1 Egg stamping

Mandatory NSW egg stamping requirements were introduced in November 2014 under the National Primary Production and Processing Standard for Eggs and Egg Products (NSW Food Authority, 2021a). All eggs sold in NSW (except as indicated below) must be individually stamped with the producer's unique identifier, usually a number or code. This helps food safety authorities trace eggs back to the farm from their point of sale. It provides a safeguard in the event of a food poisoning incident or disease outbreak. Eggs can be stamped at the farm where they are produced or at a grading facility. An exemption from stamping applies to small egg farmers that produce less than 20 dozen (240) eggs/week and, either:

- sell those eggs direct from the farm gate, or
- use those eggs for a fundraising activity where the eggs will be cooked

Businesses supplying eggs interstate will need to research the stamping requirements of the destination.

1.3.2 Non-regulatory measures

Government non-regulatory measures generally take the form of guidance documents that assist industry to comply with legislative requirements. For example, NSW DPI has developed *Requirements for small egg farms* (NSW Food Authority, 2017) and *Egg cleaning procedures* (NSW Food Authority, 2015a) factsheets to provide guidance for industry compliance with the Code and the Food Regulation 2015 (NSW).

The industry services body for egg producers and processors, Australian Eggs, has developed an extensive range of guidance and education material, including an online *Salmonella* risk assessment toolkit (Australian Eggs Limited, 2021g), a guide for producers on *Salmonella* Enteritidis (Australian Eggs Limited, 2021f) and a *Salmonella* Enteritidis operational response plan (Australian Eggs Limited, 2020b).

1.3.3 Biosecurity

1.3.3.1 Regulatory measures

The Primary Production and Processing (PPP) Standards (Chapter 4) contain food safety, hygiene and handling requirements applying to primary production and processing businesses, such as farms. The Chapter 4 standards do not address biosecurity, animal welfare or market access risks, as FSANZ does not have legislative responsibility for those aspects of primary production (for a review see (FSANZ, 2021a)).

The biosecurity requirements that apply to egg producers and processors generally cover a wider range of elements on-farm. In relation to egg production and processing, the biosecurity and food safety systems overlap in a number of areas, including bird health and a common goal of preventing the introduction and spread of disease. However, biosecurity requirements generally place a stronger focus on poultry health, nutrition, welfare, movement and breeding, in order to prevent and control the spread of disease within the flock.

Each jurisdiction in Australia has a biosecurity regulatory framework. The Australian Government, New South Wales (NSW), Queensland (QLD), Western Australia (WA) and Tasmania (TAS) have enacted specific biosecurity legislation. In other jurisdictions, biosecurity requirements are implemented through livestock and animal disease legislation. In most jurisdictions, the enforcement of biosecurity legislation is the responsibility of agencies that regulate primary industries. In some jurisdictions, these agencies also enforce aspects of food safety legislation.

In recent years, biosecurity requirements have evolved to control a broad spectrum of animal diseases, including SE in poultry. For example, under the *Biosecurity Act 2019* (TAS), the detection of SE in commercial poultry can result in mandatory flock de-population. Similarly, SE in poultry is a notifiable disease under the *Livestock Diseases Control Act 1994* (VIC). In NSW, SE is listed as notifiable in Schedule 1 of the Biosecurity Regulation 2017 under the *Biosecurity Act 2015*. The notifiable listing requires people to notify any suspect or known cases of SE to NSW Local Land Services (LLS) or to NSW DPI, within one working day of first suspecting or becoming aware of SE in poultry.

Other biosecurity agencies, such as the NSW Department of Primary Industries (NSW DPI), are taking additional action to control the spread of SE. In mid-2019, NSW DPI introduced the Biosecurity (*Salmonella* Enteritidis) Control Order 2019 to assist in the management of the biosecurity risk posed by the spread of SE. The order established minimum biosecurity standards for the poultry and egg industries and made them legally enforceable under the *Biosecurity Act 2015* (NSW). Under a revised control order introduced in mid-2020, licensed egg producers are also required to regularly test for SE. Other areas covered by the order include signage, control of persons entering premises, handwashing, disposal of dead birds, vermin control and record keeping. The Control Order in NSW is in effect until the 30th of June 2024.

1.3.3.2 Non-regulatory measures

Industry bodies and governments have produced a number of national biosecurity plans, guidelines, codes of practice and other tools and materials for egg producers. Key examples are the *National farm biosecurity technical manual for egg production* (Animal Health Australia, 2020), the *National SE Response Management Plan* (Animal Health Australia, 2021) and the *Code of practice for biosecurity in the egg industry* (Grimes & Jackson, 2015).

The *National farm biosecurity technical manual for egg production* (Animal Health Australia, 2020) applies to the biosecurity requirements for commercial table egg production farms (layer farms) from the time of preparation of the shed for chick placement to the delivery of day old chicks until depopulation of the spent layer hens, including transportation and delivery of point of lay pullets. The Manual provides practical guidance for managing key biosecurity concerns on an egg farm including training and operational procedures, facility cleaning, movement of people through the farm, management of water, managing free range areas and rodent and vermin control. It also covers the transport and movement of eggs and egg products to other farms, grading and processing establishments. It stipulates the minimum biosecurity measures for emergency animal diseases[2] on egg production farms. For the control and prevention of endemic diseases on egg production farms other enhancements to these minimum standards may be required and must include such things as vaccination and may include medication. While not specifically addressed in the manual, biosecurity measures in place on breeder farms would generally be much more stringent, reflecting the economic importance and the extended life cycle of breeder flocks (DAFF, 2009). A *National SE Response Management Plan* was also recently prepared for government and industry (Animal Health Australia, 2021). The purpose of the document is to provide best practice guidance for affected stakeholders in the event of suspected or confirmed SE linked to poultry in Australia, and knowledge of the various response networks that may be activated.

The *Code of practice for biosecurity in the egg industry* (Grimes & Jackson, 2015) has been developed by Australian Eggs Ltd. The Code aims to assist egg farmers to develop and adopt an appropriate Biosecurity Plan, based on hazard analysis and critical control point (HACCP) principles, for their started pullet and egg layer farms. The Code recommends appropriate HACCP-based Biosecurity Programmes/Procedures and Good Management Practices (GMPs) to prevent the occurrence of endemic and emergency diseases in layer and pullet flocks, the multiplication of

[2] A disease that is (a) exotic to Australia or (b) a variant of an endemic disease or (c) a serious infectious disease of unknown or uncertain cause or (d) a severe outbreak of a known endemic disease, and that is considered to be of national significance with serious social or trade implications.

pathogens on farm and their subsequent spread from farms into the environment or to other poultry. The scope of the Code extends from shed setup through to production of started pullets and fresh whole eggs.

1.4 Updating the 2013 Risk Assessment

This Risk Assessment was produced following a literature review for issues related to egg and egg products that have impacted egg product food safety since 2013. Information sources included published reports on the following:

- foodborne illness reports and recall data in Australia attributed to egg and egg products
- international issues arising from human illness or perceived hazards linked with egg and egg products
- risk assessments of egg and egg products
- emerging issues in the farm to consumer continuum for egg and egg products relevant to health risk
- research findings related to hazards in egg and egg product production and processing
- baseline surveys of microbiological and chemical hazards in egg and egg products
- other relevant sources if identified during the above activities

The current Risk Assessment includes discussion of egg and egg products identified from the literature review conducted as detailed above.

2. Risk assessment

2.1 Hazard identification

2.1.1 Biological hazards

2.1.1.1 *Salmonella*

Salmonella is usually associated with foodborne illness outbreaks involving eggs and egg products. Most cases of salmonellosis are mild; however, sometimes it can be life-threatening. The severity of the disease depends on 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 infections.

Salmonella enterica serovar Typhimurium (ST) and *Salmonella enterica* serovar Enteritidis (SE) are the two most frequently identified causes of egg-associated disease in industrialised countries (Moffatt et al., 2016). Investigations of salmonellosis outbreaks have estimated a wide range in the dose of organisms responsible for causing disease (for a review see (FSANZ, 2013)). The infectious dose has been reported to vary from <10 to 10^9 and is dependent on several factors, including the health status of the host, *Salmonella* serovar and the properties of the food vehicle. Numerous studies have reported that very low doses of *Salmonella* are sufficient to cause human salmonellosis, particularly in high-fat content and semi-solid state foods that tend to protect *Salmonella* from exposure to gastric acid. In the investigation of an SE outbreak in the United States of America (USA) in 1994 involving a nationally distributed brand of ice cream, the highest level of product contamination was only six bacterial cells per half-cup (65g) serving (Hennessy et al., 1996). It was concluded that as ice cream is distributed, stored, and eaten in a frozen state, the concentration of SE measured in the ice cream was likely that which was ingested by consumers. Similar findings resulted from the investigation of an outbreak which occurred in Norway and Finland in 1987, involving ST contaminated chocolate produced by a Norwegian company. It was reported that there were only ≤10 ST cells per 100g of chocolate in about 90% of the positive samples obtained from retail outlets, suggesting that an inoculum of fewer than 10 bacterial cells may have been sufficient to cause symptomatic disease (Kapperud et al., 1990). Collectively, these studies confirm that low-level contamination of foods by *Salmonella*, and thus extremely low infectious doses, can cause disease in humans.

In Australia the majority of egg-related foodborne salmonellosis is caused by ST. ST was responsible for 95% of the egg-related outbreaks in Australia from 2001-2016, whereas other serovars were only associated with one or two outbreaks each (Laura Ford et al., 2018). However, globally SE is more commonly linked to contaminated eggs. SE started spreading globally in the 1980s, as SE became endemic in the parental breeder flocks traded internationally to production farms to produce meat and eggs (for a review see (Li, He, Mann, & Deng, 2021)). In the 1980s, a simultaneous increase of SE infections linked to poultry occurred in North America, South America and Europe. By the late 1980s and the 1990s, SE had spread to the poultry production systems in Asia and Africa. The pandemic subsequently declined in the late 1990s in the USA and the United Kingdom (UK), but SE remains a substantial problem for poultry production and public health. SE did not become endemic in Australian laying flocks and the Australian egg industry was considered free of SE, likely because of strict rules on importation of animal products, including primary breeding and parent stocks (Martelli, Wales, & Davies, 2017; NSW Food Authority, 2013b). However, in September 2018, SE was detected in commercial poultry egg farms in NSW, with a small number of commercial poultry egg properties identified as infected (NSW Food Authority, 2021b). The spread of SE in Australia is considered a significant biosecurity risk, as SE has been reported to colonise the chicken oviduct more successfully than other serotypes of *Salmonella* and to contaminate table eggs by internalisation in the forming egg.

2.1.1.1.1 Egg contamination by *Salmonella*

There are over 2,600 *Salmonella enterica* serovars and many of them are routinely detected on egg farms (McWhorter & Chousalkar, 2020). *Salmonella* has both virulent (*e.g*. ST and SE) and avirulent (*e.g. S*. Sofia) serotypes. *Salmonella* serotypes also differ in their ability to cause contamination on the eggshell or in the egg contents. SE have been proposed to possess characteristics which contribute to the epidemiological association of this serotype with eggs. SE preferentially colonises the reproductive organs of the laying hen and has been proposed to harbour a specific battery of virulence factors which enables survival at 42°C in the hostile egg white compartment (for a review see (Raspoet et al., 2011)).

2.1.1.1.1.1 Gastrointestinal tract colonisation and systemic spread of *Salmonella* in chickens

The primary route of infection and transmission of *Salmonella* in chickens is via the faecal–oral route. Infection in chickens is a complex multistep process that can be broadly categorised into a few major events that include intestinal colonisation, invasion and systemic spread to internal organs such as the liver and spleen. Bacteria colonising the intestinal lumen are able to invade the intestinal epithelial cells, leading to gut colonisation. As a consequence, immune cells, more specifically macrophages, are attracted to the site of invasion and enclose the *Salmonella* bacteria. Although macrophages generally represent the front-line host defence against invading bacterial pathogens, *Salmonella* have evolved to exist and even grow while within this intracellular environment. The ability of *Salmonella* to replicate in host macrophages enables the establishment of systemic infection. Infected macrophages enable migration of *Salmonella* to the internal organs, including the reproductive organs. In addition to systemic spread, bacteria can also access the oviduct through ascending infection from the cloaca.

2.1.1.1.1.2 Horizontal and vertical routes of egg contamination

Contamination of eggs by *Salmonella* can occur by two routes, horizontal or vertical. Horizontal transmission occurs due to external egg shell contamination, whereas vertical transmission is a result of reproductive organ colonisation before shell formation.

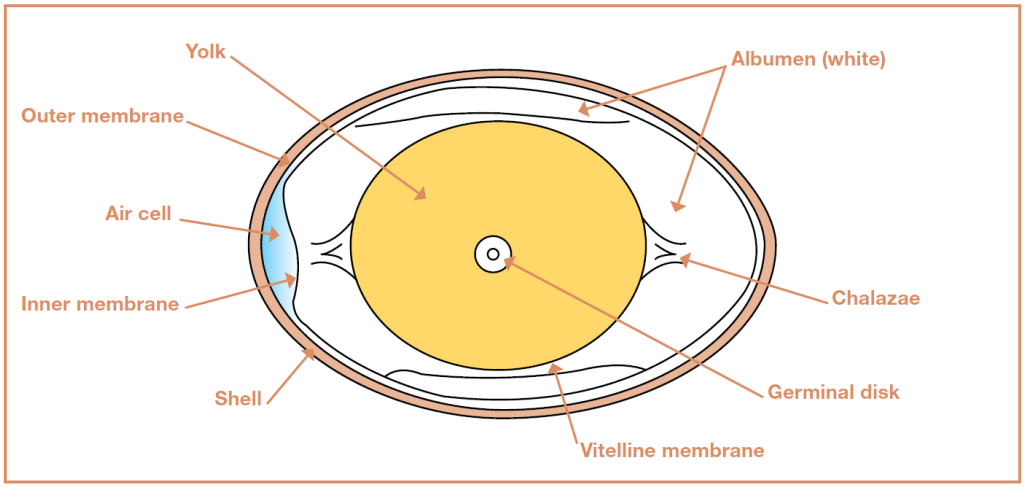
Horizontal contamination of an egg occurs when it is laid in a contaminated environment enabling bacterial adherence to the eggshell surface. Any contaminated environment in the area of the laid egg, such as the nest box, can lead to outer shell contamination. The presence of chicken manure and other moist organic materials facilitates the survival and growth of *Salmonella* by providing the required nutrients and a degree of physical protection. This highlights the importance of the rapid removal of any faecal contamination, in order to reduce the risk of externally contaminated eggs in the food chain.

Vertical transmission occurs when colonisation of the reproductive organs leads to deposition of *Salmonella* inside the edible interior contents of eggs before they are laid. *Salmonella* can be incorporated into the albumen, the eggshell membranes or the eggshell itself, depending on the site of colonisation of the oviduct. The chicken oviduct consists of five functional regions. Starting from the ovary, there are the infundibulum, magnum, isthmus, uterus and vagina. The infundibulum captures the ovulatory follicles, the magnum produces the albumen, the isthmus deposits the eggshell membranes, the uterus forms the eggshell and the vagina is involved in oviposition. Direct contamination of the yolk, yolk membranes, albumen, shell membranes and egg shell originates from infection of the chicken ovary, infundibulum, magnum, isthmus and shell gland, respectively. The hen egg presents a hostile environment for *Salmonella* (for a comprehensive overview see (Gantois et al., 2009)). If the initial site of *Salmonella* deposition is on the exterior surface of the yolk membrane or in adjacent regions of the albumen, the bacteria must be able to survive in the egg white, before reaching the egg yolk, where rapid multiplication can occur. Consequently, there is considerable interest in understanding the mechanisms underlying the ability of this organism to survive within the internal contents of eggs. The following section provides a brief overview of the structure of an egg and the physical and chemical defence mechanisms that protect its contents from microbial invasion and multiplication.

2.1.1.1.1.3 Egg structure and chemical and physical attributes

Bacteria can easily penetrate through a cracked eggshell. The intact egg, however, possesses physical barriers to bacterial penetration. These physical barriers include the shell, cuticle and membranes (Figure 1). The eggshell has small pores for exchange of gases and water vapour needed for the growth of a developing chick. These may also present a potential route for transmission of microorganisms and other materials into the egg contents. The cuticle is a hydrophobic proteinaceous layer which covers the eggshell and the pore openings. In addition to the external barriers of the shell and cuticle, inner shell membranes (separating the internal surface of the shell and the albumen) and the vitelline membrane (separating the albumen and the yolk) provide further barriers to microbial penetration.

Figure 1: Egg structure[a]



[a] Figure from (FSANZ, 2009a).

Eggs contain an arsenal of defensive systems to resist bacterial infection, many of which reside in the albumen (egg white) (for a review see (Legros et al., 2021)). Several hundred proteins have been identified in egg white and a number of these have confirmed antimicrobial properties. Some, such as lysozyme and defensins, cause damage to the bacterial envelope. Others act by inhibiting bacterial proteases (ovostatin, cystatin, ovalbumin X) or by limiting the availability of key nutrients (*e.g*. avidin acts as a vitamin chelator, forming a complex with biotin). Egg white also includes substantial quantities of the metal-chelating protein ovotransferrin. It is generally accepted that iron deficiency, which results from the strong iron-binding activity of ovotransferrin, is the key process in the defence of egg white against microbial invasion. Iron is essential for all forms of life, including bacteria, since it is involved in many essential cellular processes. Although antibacterial proteins have been identified mainly in the egg white, proteins with well-known antibacterial properties have also been associated with the eggshell and shell membranes. For example, lysozyme is abundant in the cuticle of the eggshell and the inner and outer membranes. Ovotransferrin has also been identified in the eggshell membranes and the basal calcified layer. While the vitelline membrane contains lysozyme, ovalbumin, ovotransferrin, ovomucin, lysozyme and defensins.

Other parameters also play a role in the passive immunity of egg white (Legros et al., 2021). The high viscosity of egg white can limit bacterial mobility and accessibility to nutrients, including iron. At room temperature over the 2–3 days post-laying period, the egg white pH also increases from 7.8 to 9.3, at which ovotransferrin has an enhanced ability to chelate metal ions. This pH increase is caused by the loss of CO_2 through the pores of the eggshell. The maintenance of intracellular pH around its optimum value (7.4 to 7.8) is essential for many biological functions, particularly for bacterial enzymatic activities and the status of membranes. Excessive differences in pH between the environment and the bacterial cytoplasm can lead to energetically unfavourable conditions for growth.

For microorganisms to travel to the yolk and multiply, they must traverse these physical barriers and tolerate the hostile conditions of the albumen. The bacteria that manage to reach the egg yolk are rewarded by ready access to a pool of nutrients and consequently enjoy rapid growth if the temperature is permissive.

2.1.1.1.1.4 *Salmonella* Enteritidis and eggs

In intravenously infected laying hens, SE has been reported to have a greater ability to colonise avian reproductive organs and contaminate eggs than other zoonotic serovars (e.g. ST, *Salmonella* Infantis (SI), *Salmonella* Hadar (SH), *S*. Heidelberg, *Salmonella* Montevideo) (Okamura et al., 2001). For non-SE *Salmonella* serovars, the primary route of internal contamination of the egg is considered to be via transmission through the shell. The ability of *Salmonella* to migrate into the egg contents is influenced by many factors including the integrity of the shell, cuticle and membranes, the presence and load of external contamination, differences in temperature between the egg and the environment and, humidity.

While SE grows slowly and to a limited extent in the albumen, once reaching the yolk, very rapid growth can occur under permissive temperatures (for a review see (DeWinter, Ross, Couture, & Farber, 2011)). SE is thought to gain access to the yolk over time, as the viscosity of the albumen decreases and the permeability of the vitelline membrane increases. Higher temperatures and longer storage times generally favour loss of membrane integrity and SE crossing the vitelline membrane before the egg is consumed. If the egg temperature is maintained at a sufficiently low level for the entire period of time following loss of membrane integrity, up to the point of consumption, SE is not expected to grow. The minimum growth temperature is thought to be between 6°C and 8°C and is likely to be egg and SE strain dependent. Membrane breakdown followed by storage above the minimum growth temperature can result in growth over time to a maximum population of 10^{10} organisms per egg.

Humphrey et al. (1991) undertook one of the few studies to date, to enumerate the number of SE in the contents of naturally contaminated eggs (T. J. Humphrey, Whitehead, Gawler, Henley, & Rowe, 1991). Eggs were obtained from naturally infected commercial flocks and outbreak investigations and were stored at ambient temperature (~21°C) for over 21 days. Thirteen (0.4 %) of 3,659 eggs stored for up to 21 days were SE-positive; none of which contained more than 20 cells of SE. After 21 days of storage twelve (0.5%) of 1,603 eggs were SE-positive, with five eggs containing more than 100 cells. All five eggs that were heavily contaminated (> 100 cells per egg) were more than two weeks old. Of these, only two eggs were found to contain more than 1,000 cells of SE (1.5×10^4 and 1.2×10^5 cells per egg). The site of contamination was not identified in the two most heavily contaminated eggs, but Humphrey et al. (1991) state that the results strongly suggested that it was likely to be the albumen. This was supported by the fact that the vast majority of SE-positive eggs in their study contained less than 20 cells and albumen was the principal site of contamination in 12 of 15 eggs where the yolk and albumen were cultured separately. Humphrey et al. (1991) state that their results appear to demonstrate that during the passage of the egg through the oviduct the albumen was seeded with a few cells of SE. The SE remain dormant, even in eggs stored at ambient temperature, for two to three weeks. During that time physical and chemical changes take place in the egg contents, including nutrients and possibly other factors leaking out from the yolk and negating the inhibitory properties of the albumen. In the area of the albumen closest to the yolk the growth promoting factors reach a sufficiently high concentration to permit the growth of SE and to support large populations of this organism.

2.1.1.2 Antimicrobial resistant organisms

In their Annual Report on Emerging and Ongoing Issues, FSANZ recognise antimicrobial resistance (AMR) as an ongoing food safety issue (FSANZ, 2019e). The development of AMR and emergence of multidrug resistant pathogens are global concerns for both public health agencies and the agri-food industry. Antimicrobial resistant pathogens increase the risk of an infected individual suffering an adverse health effect, such as reduced treatment efficacy, and increased disease severity, hospitalisation and mortality. Australia has strict regulations regarding

antimicrobial use in livestock. Fluoroquinolones, colistin and 4th generation cephalosporins have never been registered for use in Australian food-producing animals, gentamycin use is banned and 3rd generation cephalosporin usage remains restricted (APVMA, 2017).

FSANZ is a member of the Australian Strategic and Technical Advisory Group on AMR (ASTAG) and continues to be engaged in activities consistent with and complementary to the overall Australian Government effort to contain AMR (DoH, 2020). The 2020 Strategy (DoH, 2020) builds on the original 2015 strategy, broadening its ambit to encompass food, the environment and other classes of antimicrobials such as antifungals and antivirals.

The Australian government recently published a review of published and grey literature on AMR in food (DoH, 2018). The aim of this study was to review published and grey literature on the presence and extent of AMR in food in Australia and NZ for the period 1999 to early 2018. The report provided an overview of available evidence for AMR presence in the food production, processing and retail sectors of red meat, pork, poultry meat, dairy, egg, seafood and horticultural products. In regard to the Australian egg industry, the available AMR literature and data were assessed for information on AMR in layer pathogens, layer commensals and layer zoonotic pathogens. A limited number of studies were identified which focussed on AMR in *Salmonella* (DoH, 2018; Pande, Gole, McWhorter, Abraham, & Chousalkar, 2015; Veltman et al., 2021). No data was identified for indicator commensal bacteria such as *E. coli* and *Enterococcus* (DoH, 2018). Pande et al. (2015) examined the AMR of *Salmonella* isolates (*n*=145, 7 serovars) isolated from the dust, egg belt, faeces and shell wash from 33 commercial caged layer flocks sourced from a total of 13 farms from NSW (10 farms) and SA (3 farms). Antimicrobial susceptibility testing was conducted using the broth microdilution method and the results were interpreted according to the established Clinical and Laboratory Standards Institute (CLSI) guidelines. In cases where CLSI breakpoints were absent, results were evaluated according to the National Antimicrobial Resistance Monitoring guidelines or Swedish Veterinary Antimicrobial Resistance Monitoring guidelines. The majority of *Salmonella* isolates (133/145; 91.72%) were susceptible to all 12 antimicrobials tested in the study and no resistance was observed to fluoroquinolones or extended spectrum cephalosporins which are commonly used for the treatment of human salmonellosis. Limited resistance was observed to amoxicillin and ampicillin (5.51%), tetracycline (4.13%), cephalothin (2.06%) and trimethoprim (0.68%). None of the isolates were resistant to cefotaxime, ceftiofur, ciprofloxacin, chloramphenicol, gentamycin, neomycin or streptomycin. No multidrug resistant phenotypes (resistance to more than three classes of antimicrobial agents) were identified from any *Salmonella* isolate included in this study. All 145 *Salmonella* isolates were screened for a total of 20 antimicrobial resistance genes and the presence of class 1, 2 and 3 integrons was investigated. A low frequency of *Salmonella* isolates (4.83%) harboured antimicrobial resistance genes and a class 1 integron. The most commonly detected AMR genes among the *Salmonella* isolates were bla_{TEM} (2.07%) which confers resistance to ampicillin, *tet A* (1.38%) which confers resistance to tetracycline and, the *dhfrV* (0.69%) gene which confers resistance to trimethoprim. Ampicillin, tetracycline and trimethoprim are classified as of low importance in Australia in regard to the treatment of infections in humans, and the seriousness of the consequences of emergence of resistance (ASTAG on AMR, 2018). The authors concluded that overall, *Salmonella* isolates exhibited a low frequency of AMR and represent a minimal public health risk associated with the emergence of multidrug resistant *Salmonella* from the Australian layer industry. The second study included in the DoH (2018) review by Veltman et al. (2018), also reported an absence of resistance to highest priority critically important antibiotics (CIAs) as well as an extremely low level of AMR generally among Australian commercial egg layer *Salmonella* isolates. *Salmonella* isolates (*n*=307) from Australian commercial layer flock environments (2015–2018) were obtained from reference, research and State Government laboratories from six Australian states. The 307 isolates included 77 from NSW, 76 from QLD, 78 from Victoria (VIC), 25 from SA, 29 from TAS and 22 from WA. Isolate submission information did not disclose the layer flock production environment (caged, barn or free-range system) and it was assumed that based on production figures at the time, approximately 50% of the 307 isolates were derived from alternate systems (barn and free range). All *Salmonella* isolates were serotyped and 30 different serotypes were detected. Three key serotypes were identified that comprised approximately one third of all

isolates; ST (61/307; 19.9%), *S*. Senftenberg (45/307; 14.7%) and *S*. Agona (37/307; 12.1%). Antimicrobial susceptibility testing for 16 antimicrobial agents was performed by broth microdilution. MICs were interpreted according to CLSI guidelines and/or by using the recommended European Committee on Antimicrobial Susceptibility Testing (EUCAST) epidemiologic cut-off values (ECOFFs). CLSI breakpoints were used where animal species antimicrobial agent combinations were not available. Where no EUCAST or CLSI interpretative criteria were available, breakpoints were harmonised with those of the National Antimicrobial Resistance Monitoring System (NARMS, USA). Test results showed that 95.4% (293/307) of isolates were susceptible to all tested antimicrobial agents and all isolates were susceptible to amoxicillin-clavulanate, azithromycin, ceftiofur, ceftriaxone, ciprofloxacin, colistin, florfenicol, gentamicin, kanamycin and trimethoprim-sulfamethoxazole. A low percentage of isolates (4.6%) were non-susceptible to one (3.2%; *n*=10), two (0.7%; *n*=2) or three (0.7%; *n*=2) antimicrobial classes. Low levels of nonsusceptibility were observed to streptomycin (2.3%, *n*=7), sulfisoxazole (2.0%, *n*=6), chloramphenicol (1.3%, *n*=4) and tetracycline (1.0%, *n*=3). Very low levels of non-susceptibility were observed to ampicillin (0.7%; *n*=2) and cefoxitin (0.7%; *n*=2). Isolates showing non-susceptibility to one or more antimicrobial agents in three or more antimicrobial classes were classified as MDR. MDR isolates and ST exhibiting dual resistance were further characterised by whole genome sequencing (WGS) in order to identify the resistance genotype responsible for the phenotype. Two isolates (*S*. Havana and *S*. Montevideo), exhibited MDR phenotypes to streptomycin, sulfisoxazole and tetracycline. WGS revealed that *S*. Havana and *S*. Montevideo both possessed three of the same genes involved in streptomycin resistance (*aadA4*, *sul1*, *tetB*) and additionally, *S*. Havana also possessed the *aac*(*6'*)-*Iaa* gene involved in streptomycin resistance. One ST isolate was resistant to ampicillin and tetracycline and possessed both *tetA* (tetracycline resistance) and $bla_{\text{TEM-1B}}$ (ampicillin resistance).

Outside of the 1999 to early 2018 period covered in the literature review of the report by the Department of Health (DoH, 2018), there have been a couple of AMR studies relating to the egg industry (Sodagari et al., 2019; Sodagari et al., 2021). These studies are briefly described below and of note, Sodagari et al. (2021) report the isolation of non-wild-type resistance to fluoroquinolones in *E. coli* isolated from supermarket eggs in WA.

Sodagari et al. (2019) investigated the occurrence of AMR in *Salmonella* isolated from retail egg samples in WA (Sodagari et al., 2019). A total of 200 visually clean and intact retail egg samples (each containing a dozen eggs) were purchased for one year (2017–2018) from supermarkets in metropolitan Perth. For each sample, the contents and shells of the 12 eggs were separately pooled and cultured according to standard methods. Overall, *Salmonella* was detected in 11.5% (23/200) of the tested egg samples. *Salmonella* was isolated from 4.5% (9/200) and 3% (6/200) of eggshells and egg contents, respectively. In 4% (8/200) of the samples, *Salmonella* was recovered from both eggshell and egg contents. Isolates from positive retail egg samples were serotyped as either ST (52.2%; 12/23) or SI (39.1%; 9/23). Isolates of both ST (*n*=29) and SI (*n*=12) from *Salmonella*-positive retail packs (*n*=23) were retained for further characterisation (*n*=41). Antimicrobial susceptibility testing was performed by micro-broth dilution using commercially prepared panels. The panel comprised of 14 antimicrobials: ampicillin, azithromycin, cefotaxime, ceftazidime, chloramphenicol, meropenem, nalidixic acid, ciprofloxacin, tetracycline, tigecycline, colistin, gentamicin, trimethoprim and sulfamethoxazole. Minimum inhibitory concentrations (MICs) were interpreted using ECOFFs according to the criteria set by the European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015. ECOFFs are not a predictor of clinical success, rather measures of an antimicrobial drug MIC distribution that separate bacterial populations into wild type and non-wild type populations that exhibit an acquired or mutational resistance. Only two (ST) isolates showed resistance to ampicillin, of which one carried the β-lactamase resistance gene $bla_{\text{TEM-1B}}$. The remaining isolates (39/41) were susceptible to all 14 antimicrobials included in the MICs testing panel.

Sodagari et al. (2021) investigated the occurrence of AMR in *E. coli* isolated from caged and non-caged retail table eggs in WA. A total of 2172 visually clean and intact retail eggs were purchased between October 2017 and June

2018 from retail supermarkets belonging to five major retailers from different branches across Perth. The proportion of samples from each type of production system was targeted at approximately 50% free-range eggs, 30% cage eggs and 20% barn-laid eggs. A single carton containing a dozen eggs was considered as a single sample, resulting in a total of 181 (2172/12) samples. *E. coli* was detected in 36 out of 181 retail egg samples (19.8%). Of the 36 positive samples, *E. coli* was detected either on the shells in 34 samples or in the content of two samples (positive shells and content come from different samples). *E. coli* was recovered from 20.3% (12/59) of cage eggs, 20.0% (7/35) of barn eggs, and 19.5% (17/87) of free-range eggs pooled samples. There were no statistically significant differences ($P > 0.05$) between *E. coli* recovery from samples of the three different production systems. Three isolates (out of five confirmed) from each of the 36 *E. coli* positive egg samples were retained for further antimicrobial screening. One hundred isolates were selected, comprised of a similar ratio to represent the initial sample size of the three different housing systems, with 49 isolates from free-rage, 19 from barn-laid and 32 isolates from cage eggs. The 100 *E. coli* isolates were characterised for their phenotypic antimicrobial resistance using MIC. Antimicrobial susceptibility testing was undertaken using micro-broth dilution against a panel of 14 antimicrobials: ampicillin, azithromycin, cefotaxime, ceftazidime, chloramphenicol, meropenem, nalidixic acid, ciprofloxacin, tetracycline, tigecycline, colistin, gentamicin, trimethoprim and sulfamethoxazole. MICs were interpreted using ECOFFs indicated in the EUCAST. Isolates were classified as multi-class resistant (MCR) if they exhibited MICs above the ECOFFs for one or more antimicrobial agents in three or more antimicrobial classes. Fifty-seven (57%) of the recovered *E. coli* isolates were resistant to at least one of the 14 antimicrobials included in the MIC testing panel, of which 22 isolates (22%) showed multi-class resistance. Out of the characterised 100 *E. coli* isolates, the highest frequencies of non-wild type isolates were found against tetracycline (49 isolates), followed by ampicillin (36 isolates), trimethoprim (20 isolates), sulfamethoxazole (18 isolates), and ciprofloxacin (two isolates). According to EUCAST, there is no ECOFF available for the combination *E. coli*/Azithromycin. However, two isolates demonstrated an MIC for azithromycin which was above the CLSI M100 clinical resistance breakpoints for *Enterobacteriaceae*. The results indicate that 57% of the isolates showed MICs above ECOFF value for at least one antimicrobial agent with significantly lower ($P < 0.001$) frequency among *E. coli* isolated from cage eggs (34.3%; 11/32) compared with those from free-range (69.3%, 34/49) and barn-laid (68.4%, 13/19) eggs. Of the MCR *E. coli* isolates, the majority (16/22, 72.7%) were from free-range positive egg samples. The authors conclude that their results indicate that resistance to some of the CIAs in human use is rare among generic *E. coli* isolated from WA retail eggs. However, notable levels of resistance to antimicrobials with less critical ratings, such as tetracycline (49%) and ampicillin (36%) were observed. A subset of *E. coli* isolates (n=14) were selected for further characterisation using WGS, based on their AMR and MCR patterns against some of the clinically important antimicrobial classes utilised in human medicine, including fluoroquinolones and macrolides. WGS revealed that *tet*(A) and $bla_{\text{TEM-1B}}$ genes were present in most of the isolates exhibiting phenotypic resistance to tetracycline and ampicillin, respectively. Two isolates demonstrated reduced susceptibility to ciprofloxacin (a fluoroquinolone) and WGS revealed that both isolates harbour *qnrS1*-bearing IncX plasmids. While the occurrence of *qnr* genes have been actively investigated worldwide, their clinical significance has not been thoroughly investigated (Sodagari et al., 2021). Ciprofloxacin is classified as of high importance in Australia in regard to the treatment of infections in humans, and the seriousness of the consequences of emergence of resistance (ASTAG on AMR, 2018). The authors report that this is the first detection of non-wild-type resistance to fluoroquinolones in supermarket eggs in Australia; with one of the two isolates was from a cage-laid eggs sample while the other was from a barn-laid retail eggs sample. Fluoroquinolones have never been permitted for use in poultry farms in Australia. The authors conclude that the detection of low-level ciprofloxacin-resistant *E. coli* in the absence of local antimicrobial selection pressure at the Australian layer farms warrants further research on the potential role of the environment or human-related factors in the transmission of AMR.

While Australia has one of the most conservative approaches in the world to the use of antimicrobials in food producing animals (DoH & DAWE, 2020), industry and government need to continue to proactively monitor AMR and

antimicrobial stewardship practices to ensure the long-term protection of both animal and human health. The NSW Government plays an established role in antimicrobial stewardship and resistance in accordance with the National Antimicrobial Resistance Strategy. Within scope of the DPI and LLS is antimicrobial stewardship and antimicrobial resistance in terrestrial livestock, bees, aquatic animals, companion animals, pet shops and wildlife (NSW Government, 2018). Effective vaccination programs and strict biosecurity measures limit the occurrence of endemic diseases and reduce the requirement for antimicrobial treatments in layer flocks. There is no current role for the NSW Food Authority beyond its existing role in promoting good hygienic practices to combat the foodborne transmission of bacteria with AMR.

2.1.2 Chemical hazards

The egg food safety scheme specifies that eggs for sale are to be free of chemical contaminants. An egg producer must not sell eggs for human consumption that have been obtained from a bird that has been administered a veterinary chemical product (within the meaning of the Agvet Code) in contravention of the *Stock Medicines Act 1989* or the *Pesticides Act 1999* unless the NSW Food Authority has approved in writing of the sale of the eggs. Agvet Code means the provisions applying because of section 5 of the *Agricultural and Veterinary Chemicals (NSW) Act 1994*.

The National Residue Survey (NRS) program monitors the levels of, and associated risks from, pesticides and veterinary medicine residues and contaminants in Australian food products [for an overview see (DAWE, 2021)]. NRS supports Australia's primary producers and food processors in producing products which meet both Australian and relevant international standards. Exporters of animal products are required under Australian law to participate in the NRS program, while non-exporting animal product industries use the NRS on a voluntary basis to demonstrate compliance with state food safety obligations. NRS programs cover a range of commodities, including hen eggs. Export volume data was reported by Australian Eggs Limited for the 2019-20 financial year for shell eggs (237.8 mt), egg pulp/liquid (485.2 mt) and egg powder (22.3 mt) (Australian Eggs Limited, 2020a). Of the 203 hen eggs sampled in 2019–20 as part of the NRS program, there was a 100% compliance rate relative to Australian standards for a range of antibiotics, anticoccidials, contaminants, insecticides and metals (DAWE, 2020b). While the NRS program reports data representing a small part of the broader Australian egg production industry sector within Australia, this result indicates that chemical hazards are well controlled at primary production under existing regulatory and non-regulatory measures.

2.1.3 Physical hazards

Physical contaminants associated with egg and egg products include intrinsic contaminants (e.g. those introduced through malfunctioning of the shell glands) and extrinsic contaminants (e.g. material that is foreign to the nature of the food such as metal, glass, plastic). Extrinsic physical hazards are mainly a concern for egg products and may be introduced at any stage of the processing chain such as via raw materials, poorly maintained facilities and equipment, packaging materials and poor food safety practices. Physical hazards would normally be addressed by adherence to GMPs, a HACCP system and requirements relating to safe and suitable food in Chapter 3 of the Code. Physical hazards are less likely than chemical or biological contaminants to affect large numbers of people and, are most likely to be reported by production or by consumer complaints. There have been no recent consumer level recalls in Australia of egg or egg products due to the presence of physical objects (see Section 2.3.4).

2.2 Exposure assessment

2.2.1 Consumption of eggs and egg products

Australian egg consumption data is summarised below from the 25th Australian Total Diet Study (ATDS) (FSANZ, 2019c) in Table 1, from the Australian Bureau of Statistics (ABS) (ABS, 2020a) in Table 2 and, from Australian Eggs Limited (Australian Eggs Limited, 2021a) in Table 3. Assuming an average egg weighs 58 grams (Australian Eggs

Limited, 2021a), the mean daily egg consumption per person reported in the 25th ATDS (FSANZ, 2019c) and by the ABS (ABS, 2020a) would equate to a lower number of eggs consumed annually per person than that reported by Australian Eggs Limited (Australian Eggs Limited, 2021a). The difference in the values reported by each source may be explained by the different methodologies employed. As stated in the ATDS (FSANZ, 2019a), participants in 24-hour food recalls may over, or under, report food consumption. Typically, data derived from 24-hour food recall surveys can lead to conservative assessments, particularly where exposure arises from the consumption of non-habitually eaten foods (FSANZ, 2019a). The data estimates reported by the ABS (ABS, 2020a) were comprised of two components, consisting of aggregated sales data provided by the major retailers and, for sales outside of the major retailers estimates were based on household expenditure data (ABS, 2020b). Overall, data released by Australian Eggs Limited shows that average consumption in Australia has continued to increase from 2014-15 FY to 2018-19 FY, with average consumption reported to be 247 eggs per person per year in 2018-19 FY (Australian Eggs Limited, 2021a).

Table 1: Egg consumption data for Australian consumers (2 years and above)[a]

Food group	Mean food consumption amount for respondents[b] (grams per person per day)	Mean food consumption amount for consumers[c] (grams per consumer per day)	Proportion of population consuming food groups (%)
Eggs	14	24	59

[a] Data reported in the 25th ATDS (FSANZ, 2019b).

[b] Respondent – Any person included in a nutrition survey, irrespective of whether they are reported consuming a particular food of interest or not.

[c] Consumer – A respondent in a nutrition survey who reports consuming a particular food within the previous 24 hours.

Table 2: Apparent consumption of egg products and dishes in Australia[a]

Food group	Mean daily per capita consumption (grams)	
	2018 - 19	2019 - 20
Eggs	18.6	19.6
Dishes where egg is the major ingredient	0.1	0.0

[a] Data reported by the ABS (ABS, 2020a). The 'Egg products and dishes' food group includes eggs and dishes where eggs are the major component e.g. omelettes, frittatas and souffles.

Table 3: Egg consumption per person in Australia[a]

Year	Eggs consumed
2018-19 FY	247 eggs per person, per year
2017-18 FY	245 eggs per person, per year

Year	Eggs consumed
2016-17 FY	231 eggs per person, per year
2015-16 FY	226 eggs per person, per year
2014-15 FY	221 eggs per person, per year

[a] Data obtained from the Australian Eggs Limited website (Australian Eggs Limited, 2021a).

2.3 Hazard characterisation

2.3.1 Overview of foodborne illness and egg and egg products in NSW from 2013 to 2020

The previous risk assessment (NSW Food Authority, 2013b) included discussion of OzFoodNet reports spanning 2009 to March 2012, as well as review of the foodborne illness investigations conducted by the NSW Food Authority in the period from 2009 to November 2012. The current risk assessment includes discussion of foodborne illness outbreaks in NSW from 2013 to 2020.

Table 4 displays a summary of the total number of foodborne or potentially foodborne disease outbreaks investigated in NSW from 2013 to 2020, as well as the number of these outbreaks in which eggs; alone or in a complex food, were identified as the suspected or responsible vehicle (Communicable Diseases Branch, 2014, 2015, 2016, 2017, 2018, 2019, *In print-a*, *In print-b*). As can be seen in Table 4, on average the suspected/responsible food vehicle was identified in only a minority of outbreaks from 2013 to 2020 (43% ± 14%). A possible explanation for this is the delay between consumption of foods and reporting of illness, which impairs case recall of foods and ingredients consumed (Communicable Diseases Branch, 2015). This also reduces the ability of the NSW Food Authority to obtain specimens of implicated foods and timely environmental samples (Communicable Diseases Branch, 2015). In addition, not all reported outbreaks can be properly investigated due to factors such as lack of cooperation from cases (an outbreak is often reported by one case, representing many cases who may not want to collaborate) and prioritisation of resources (Communicable Diseases Branch, 2015). It is therefore acknowledged that the role of various food commodities as vehicles of foodborne disease may be underestimated.

Eggs; alone or in a complex food, were identified as the suspected or responsible vehicle in a total of 52 outbreaks from 2013 to 2020 (Table 4). Two to 15 outbreaks were linked to eggs or egg-related dishes each year from 2013 to 2020 (Table 4).

The term "*raw*" and "*undercooked*" were used to describe the suspected or responsible vehicle in 48% (25/52) and 17%% (9/52) of cases, respectively. Cross contamination was implicated in 15% (8/52) of cases.

Where the suspected or responsible vehicle was reported, multiple outbreaks were linked to eggs (31%; 16/52) and undercooked eggs (10%; 5/52). Of those outbreaks where the suspected or responsible vehicle was reported and involved a complex food, multiple outbreaks were linked to raw egg mayonnaise (10%; 5/52), other raw egg sauces *e.g. aioli, béarnaise* (10%; 5/52), Vietnamese rolls (10%; 5/52), tiramisu (8%; 4/52), fried ice-cream (6%; 3/52) and raw egg salad dressings *e.g. caesar* (4%; 2/52).

Salmonella was the causative agent of all (100%; 52/52) egg-related outbreaks from 2013 to 2020 (Table 5). The most common serovar implicated was ST (83%; 43/52), followed by SE (14%; 7/52) and *S*. Virchow (SV) (2%; 1/52). In one of the outbreaks (2%; 1/52), the serotype was not determined. In some of the outbreaks involving ST (75%; 39/52), further characterisation of the outbreak strain was undertaken and details of the MLVA profile or phage type (PT) were

reported (for further detail see Table 5). Of particular note, the first reported locally acquired SE egg-related outbreak occurred during this time period.

Restaurants were the most common outbreak setting from 2013 to 2020 and were implicated in 50% (26/52) of all egg-related outbreaks (Table 5), followed by bakeries (15%; 8/52), private residences (14%; 7/52) and take-away venues (12%; 6/52).

Table 4*:* Summary of foodborne or potentially foodborne disease outbreaks reported in NSW from 2013 to 2020

	2013	2014	2015	2016	2017	2018	2019	2020
Total number of foodborne or potentially foodborne disease outbreaks	39	44	58	70	38	50	58	31
Number of people affected in all outbreaks	> 417	> 480	> 569	> 1,625	> 437	> 560	> 604	> 443
Percentage of all outbreaks in which the suspected/responsible vehicle was known	33% (13/39)	52% (23/44)	45% (26/58)	36% (25/70)	26% (10/38)	32% (16/50)	67% (39/58)	55% (17/31)
Total number of outbreaks in which eggs; alone or in a complex food, were specifically identified as the suspected/responsible vehicle[a]	4	9	12	4	2	4	15	2

[a] In Table 11 (page 20) of the 2013 OzFoodNet Working Group Annual Report (Communicable Diseases Branch, 2014), a column titled “responsible vehicle” lists the food item(s) for each outbreak where available, otherwise “unknown” is recorded. The evidence used to categorize the food item(s) as a responsible vehicle, are either solely or a combination of descriptive, analytical or microbiological evidence. From 2014 onwards, the OzFoodNet Working Group Annual Reports list the food item(s) for each outbreak where available as “Suspected / Responsible vehicle”, otherwise “unknown” is recorded. The evidence used to categorize the food item(s) as a “Suspected / Responsible vehicle”, is as listed above for the 2013 OzFoodNet Working Group Annual Report.

Table 5: Foodborne disease outbreaks reported in NSW between 2013 and 2020[a], in which eggs; alone or in a complex food, were specifically identified as the responsible vehicle

Year	Month of onset	Setting	Pathogen	No. affected	No. hospitalised	Suspected or responsible vehicle
2020	May	private residence	*S.* Typhimurium	4 (2 lab confirmed)	2	Eggs - ingestion of contaminated raw products
	January	private residence	*S.* Typhimurium	11 (1 lab confirmed)	0	Eggs - ingestion of contaminated raw products
2019	September	private residence	*S.* Typhimurium	17 (7 lab confirmed)	Unknown	Raw egg icing - Ingestion of contaminated raw products
	July	restaurant	*S.* Typhimurium	3 (3 lab confirmed)	0	Raw egg aioli - improper food preparation
	May	bakery	*S.* Enteritidis	3 (3 lab confirmed)	0	Raw egg mayonnaise – improper food preparation
	April	bakery	S. Typhimurium (MLVA 5-17-9-13-490)	3 (3 lab confirmed)	1	Eggs
	April	restaurant	S. Typhimurium (MLVA 5-17-9-13-490)	7 (6 lab confirmed)	2	Eggs - inadequate cleaning of equipment
	April	bakery	S. Typhimurium (MLVA 5-17-9-13-490)	6 (6 lab confirmed)	1	Eggs - inadequate cleaning of equipment
	February	commercial caterer	*S.* Typhimurium (MLVA 5-17-9-13-490)	3 (3 lab confirmed)	2	Eggs
	February	restaurant	*S.* Typhimurium (MLVA 5-17-9-13-490)	3 (2 lab confirmed)	0	Eggs
	February	bakery	*S.* Typhimurium (MLVA 5-17-9-13-490)	43 (24 lab confirmed)	6	Eggs - inadequate cleaning of equipment

Year	Month of onset	Setting	Pathogen	No. affected	No. hospitalised	Suspected or responsible vehicle
	February	take-away	*S.* Enteritidis	8 (7 lab confirmed)	5	Eggs, Vietnamese rolls - Cross contamination from raw ingredients, inadequate cleaning of equipment
	January	restaurant	*S.* Typhimurium (MLVA 5-17-9-13-490)	3 (3 lab confirmed)	2	Eggs
	January	restaurant	*S.* Typhimurium (MLVA 5-17-9-13-490)	2 (2 lab confirmed)	1	Eggs
	January	restaurant	*S.* Enteritidis	21 (12 lab confirmed)	8	Fried ice cream (eggs) – improper food handling
	January	private residence	*S.* Enteritidis	6 (1 lab confirmed)	1	Eggs
	January	restaurant	*S.* Enteritidis	14 (10 lab confirmed)	4	Eggs - cross contamination from raw ingredients, inadequate cleaning of equipment
2018	November	aged care	*S.* Typhimurium MLVA 5-17-9-13-490	17	4	Eggs - cross contamination from raw ingredients
	October	restaurant	*S.* Enteritidis	3	1	Eggs
	September	restaurant	*S.* Enteritidis	30	1	Eggs - cross contamination from raw ingredients
	September	restaurant	*S.* Typhimurium MLVA 3-16-10-17-523	17	2	Raw egg tiramisu
2017	January	restaurant	*S.* Typhimurium MLVA 3-16-9-7-523	17	0	Multiple – raw eggs and cross contamination

Year	Month of onset	Setting	Pathogen	No. affected	No. hospitalised	Suspected or responsible vehicle
	January	picnic	*S.* Typhimurium MLVA 3-17-9-12-523	9	2	French toast (with egg)
2016	December	restaurant	*S.* Typhimurium MLVA 3-18-9-11-523	6	0	Raw egg sauce
	October	restaurant	*S.* Typhimurium MLVA 3-9-7-12-523	8	0	Raw egg sauce used in multiple menu items
	October	restaurant	*S.* Typhimurium MLVA 3-10-8-11-496	4	0	Items containing undercooked eggs
	July	restaurant	*S.* Typhimurium MLVA 3-11-15-10-523	5	1	Raw egg products
2015	November	restaurant	*S.* Typhimurium MLVA 3-26-13-8-523	40	2	Undercooked egg products
	October	take-away	*S.* Typhimurium MLVA 3-12-11-14-523	5	0	Raw egg mayo
	September	bakery	*S.* Typhimurium MLVA 3-16-9-11-523	12	9	Vietnamese pork rolls, use of undercooked egg and chicken liver products & cross contamination
	September	restaurant	*S.* Typhimurium MLVA 3-12-11-14-523	40	0	Fried ice-cream containing undercooked egg
	May	restaurant	*S.* Typhimurium MLVA 3-12-12-9-523	11	0	Dessert containing raw egg
	March	restaurant	*Salmonella* PCR+	5	1	Tiramisu made with raw eggs
	March	restaurant	*S.* Typhimurium MLVA 3-9-7-13-523	4 (2 were ACT residents)	0	Menu items containing undercooked egg

Year	Month of onset	Setting	Pathogen	No. affected	No. hospitalised	Suspected or responsible vehicle
	March	community event	*S.* Typhimurium PT 12A	4	3	Pancake batter containing undercooked eggs
	February	restaurant	*S.* Virchow	3	1	Menu items containing undercooked egg
	January	restaurant	*S.* Typhimurium MLVA 3-12-11-14-523	12	0	Menu items containing undercooked egg
	January	restaurant	*S.* Typhimurium MLVA 3-12-11-14-523	2	0	Suspected raw egg sauces
	January	restaurant	*S.* Typhimurium MLVA 3-9-8-12-523	3	0	Tiramisu made with raw eggs
2014	October	take-away	*S.* Typhimurium MLVA 3-10-7-12-523	26	0	Chocolate mousse cake made with raw egg
	September	take-away	*S.* Typhimurium MLVA 3-26-13-8-523	13		Raw egg mayo
	June	private residence	*S.* Typhimurium MLVA 3-24-12-10-523 (9) and 3-24-13-10-523 (1)	13	0	Tiramisu with raw egg
	May	take-away	*S.* Typhimurium MLVA 3-10-7-12-523	11	1	Vietnamese rolls raw egg
	April	bakery	*S.* Typhimurium MLVA 3-17-10-11-523	33	7	Raw egg mayo
	April	take-away	*S.* Typhimurium MLVA 3-26-7-20-496	11	2	Raw egg salad dressing
	February	other	*S.* Typhimurium MLAV 3-10/11-7-12-523	8	2	Raw egg mayonnaise

Year	Month of onset	Setting	Pathogen	No. affected	No. hospitalised	Suspected or responsible vehicle
	February	bakery	*S.* Typhimurium MLVA 3-16-9-12-523	26	3	Vietnamese rolls with raw egg butter
	January	restaurant	*S.* Typhimurium MLVA 3-24-12-12-523	2	0	Raw egg caesar salad dressing
2013	October	bakery	*S.* Typhimurium MLVA 3-10-7-14-523	49	21	Vietnamese-style rolls. Likely raw egg mayo and cross contamination.
	June	private residence	*S.* Typhimurium MLVA 3-23-23-11-523	17	5	Béarnaise sauce
	March	private residence	*S.* Typhimurium MLVA 3-17-9-12-523	4	4	Raw egg smoothies
	February	restaurant	*S.* Typhimurium MLVA 3-9-7-14-523	7	3	Fried ice cream (undercooked egg)

[a] Data was obtained from the NSW OzFoodNet annual reports from 2013 to 2020 (Communicable Diseases Branch, 2014, 2015, 2016, 2017, 2018, 2019, *In print-a*, *In print-b*)

2.3.2 Notable foodborne illness reports

2.3.2.1 NSW *S.* Enteritidis outbreak linked to local eggs

SE was not thought to be endemic in Australia until 2018, when a slight increase in SE cases was detected in June and an outbreak occurred originating from NSW egg farms. All notified cases of SE are investigated in NSW to determine likely place of acquisition (local vs overseas) and locally acquired cases are further investigated in conjunction with the NSW Food Authority. The section below details the outbreak investigation as described in the NSW OzFoodNet Annual Surveillance Reports (Communicable Diseases Branch, 2019, *In print-a*).

Following the increase in SE cases, the investigation determined that a higher number of cases than expected reported no history of travel. All cases also either lived in or had travelled to the metropolitan Sydney region during their respective exposure periods. Results from WGS found that a portion of these infections were closely related. A breakthrough in the investigation occurred when a birthday cake was strongly implicated as the likely cause of illness among a family of cases. An uneaten portion of the cake was found to be positive for SE and closely linked by WGS to the outbreak sequence. Trace back of the various ingredients in the cake identified the eggs used to make the cake were supplied by a local licensed egg farm which supplied the metropolitan Sydney area. The NSW Food Authority inspected the egg producer and the associated egg packing facility. Environmental samples obtained during inspections of the facilities were found to be positive for SE and WGS confirmed the strain on the farm was related to the cluster of human cases. Biosecurity measures at the property were immediately implemented by the NSW DPI and the biosecurity response plan for the detection of SE in Australian eggs was activated. The NSW Food Authority

issued a product recall on 8 September 2018 for all eggs produced at the facility, sold under the brand Glendenning Farms.

Four days later, on 12 September 2018, a second cluster of illness potentially linked to a Sydney café was identified through the NSW *Salmonella* SMS Project. This project sends all notified *Salmonella* cases with a mobile phone number an SMS requesting details of any food venues where they had eaten in the three days prior to illness onset. SMS responses from two cases indicated that two school groups from separate areas of regional NSW who visited Sydney in early September had fallen ill with *Salmonella*-like illness. Both school groups stayed at the same accommodation facility and ate food provided by the venue's associated café. Thirty attendees across both school groups reported symptoms and six cases across both school groups were later confirmed as SE. One additional case was *Salmonella* positive by polymerase chain reaction (PCR) only. WGS confirmed the six infections were related to the outbreak. A cohort study was undertaken of one of the school groups and the highest attack rate implicated a chicken, lettuce and mayonnaise sandwich. The NSW Food Authority inspected the café and confirmed only a commercial mayonnaise was used on site. However, it was established that the café had been using the brand of recalled eggs onsite when the students were in Sydney. One composite kitchen surface swab was found to be positive for SE, which was found to be related by WGS to the outbreak sequence. As a result, while the school group did not directly consume eggs at the café, this cluster of illness is attributable to cross contamination in the kitchen at the time of food preparation. Despite the egg recall, locally acquired SE cases occurring among residents of, or visitors to, metropolitan Sydney continued to occur during the last quarter of 2018.

One point source cluster linked to the outbreak was investigated during the final quarter for 2018. Three unrelated cases reported dining at a yum cha restaurant on the same day in October 2018. The NSW Food Authority inspected the venue, but no evidence of the recalled eggs from September were found on site. Several food samples and swabs were collected by the NSW Food Authority and all tested negative for *Salmonella*. The venue was found to have inadequate sanitiser in use and was serving fried ice cream. A prohibition order was issued regarding the sale of fried ice cream.

Between 18 May and 31 December 2018, a total of 58 cases of this outbreak strain of SE were notified in NSW residents. In addition, infections in two residents from other Australian jurisdictions who had travelled to Sydney and, one person where travel to NSW could not be established, were linked to the outbreak by WGS. Potential exposures within the state of residence were investigated and ruled out. Investigation into the source of introduction and the spread of the infection continued into the first quarter of 2019.

Five point source clusters were linked to the outbreak during 2019, with two occurring in restaurants, two in take-away venues and one at a private residence. In the first point source cluster, at least 14 people from five unrelated dining groups became unwell after dining at an Asian restaurant in metropolitan Sydney. Ten people were tested and found to have an SE infection. Symptoms appeared between 30 December 2018 and 23 January 2019. Food history information varied and no common food items were identified between the unwell diners. The NSW Food Authority inspected the venue with the local council and identified hygiene and cross-contamination issues at the restaurant. The business was found to have poor food handling practices in place and was not using adequate sanitiser. The business voluntarily agreed to close the premises to improve handling and cleaning practices. Four food samples collected during the initial inspection returned positive for SE, including raw meat, cooked prawn, canned meat and ham. Trace-back of ingredients supplied to the restaurant led to a NSW Food Authority inspection of the supplying egg farm. This egg producer had two properties which were both subsequently found to be contaminated with SE linked by WGS to the original outbreak. A consumer advisory was issued on 1 February 2019. This egg producer was later found to have links to the first contaminated farm identified in September 2018.

Shortly afterwards, the NSW *Salmonella* SMS project identified a second point source outbreak, at a different restaurant, linked to this same egg farm. At least 21 people from six unrelated dining groups became unwell following

dining at a restaurant in metropolitan Sydney, of which 12 were confirmed to be SE. Symptom onsets occurred between 1 January 2019 and 29 January 2019. A survey was sent to 15 cases to establish any common food exposures. Of the 11 responses received, nine people reported consumption of fried ice cream (82%) and BBQ chicken (82%). The NSW Food Authority inspected the venue and found the restaurant was using raw eggs to make fried ice cream. The NSW Food Authority samples of the fried ice cream were positive for SE. A prohibition order was served on the business and the venue was closed for two weeks.

In the third point source cluster, eight people from seven unrelated dining groups became unwell after consuming take-away at a venue in metropolitan Sydney. Of these, six were confirmed to be SE, one *Salmonella* PCR positive only, and one did not submit specimens for testing. Symptom onsets occurred between 15 February 2019 and 21 February 2019. All seven *Salmonella* cases reported consuming Vietnamese rolls, of which five ate pork rolls, one ate a chicken roll, and one ate a roll with unspecified meat. NSW Food Authority conducted an inspection of the venue and found the take-away venue used eggs supplied by the producer identified in the above outbreaks. The eggs should not have been onsite. The take-away venue was issued a prohibition order.

In the fourth point source cluster, three unrelated cases of SE were identified as having consumed Vietnamese rolls from the same bakery in metropolitan Sydney. Symptom onsets ranged between 17 and 21 May 2019. All cases reported consuming either a pork, chicken or salad Vietnamese style roll containing mayonnaise. The NSW Food Authority inspected the venue and found the business was making raw egg mayonnaise and not following raw egg guidelines. Swabs and samples collected during the inspection did not return any positives for *Salmonella*. A prohibition order regarding raw egg use was issued. The egg distributor which supplied the eggs to the business was subsequently found to have positive detections of SE on site and an egg recall was issued on 14 June 2019.

Active surveillance of egg farms and grading facilities for SE by NSW DPI and NSW LLS led to the identification and confirmation of the presence of SE on several other properties. This resulted in three additional egg recalls in NSW during the second quarter of 2019: Southern Highland Organic Eggs on 6 April 2019, Steve's Farm Fresh Eggs on 16 April 2019 and Port Stephens Eggs on 7 May 2019.

On 1 August 2019, a Biosecurity Control Order was issued by DPI to prevent further spread of SE across the industry. The Control Order established minimum biosecurity standards for the poultry and egg industries and made them legally enforceable under the *Biosecurity Act 2015* (NSW).

There were no SE outbreaks detected in 2020 (Communicable Diseases Branch, *In print-b*).

In total, there were 245 *Salmonella* infections linked to the outbreak strain of SE, including 234 cases confirmed by WGS, six cases positive by *Salmonella* PCR only and five secondary infections. Of these, 190 were residents of NSW. Seven egg recalls (six in NSW and one in VIC) and one consumer advisory (in NSW) had been issued. SE was detected at a number of egg production properties and grading facilities. Section 2.3.2.1.1 provides an overview of the SE infected premises.

2.3.2.1.1 SE infected premises in NSW

As part of the response to the SE outbreak, the NSW DPI increased surveillance and monitoring at egg farms and grading facilities. If a poultry farm or facility has confirmed positive tests for SE it is quarantined under an Individual Biosecurity Direction (IBD) that is issued by NSW DPI (NSW DPI, 2021b). The IBD puts legal restrictions on the farm so that eggs, birds, other livestock, equipment and litter are only able to be removed under a permit to a licenced or approved facility. As well as limited access to and movement on affected farms, flocks are depopulated, the facilities and property decontaminated and disinfected. Extensive clearance sampling and testing was conducted on infected properties after decontamination and disinfection, to confirm that they were ready to recommence egg production. Affected properties were only permitted to recommence egg production after strict biosecurity and food safety

standards were met. Tracing of facilities or other businesses with recent contact with the affected premises was also undertaken and samples from those properties were collected and investigated.

In total, over 6,000 environmental and egg samples were taken by NSW DPI for microbiological testing as part of surveillance, monitoring and clearance activities across NSW. On farm, microbiological samples were collected from up to three sheds (two boot swabs, two sponge swabs, dust and faecal samples). At grading facilities, the microbiological samples that were collected included three boot swabs (receival area, grading room floor, egg washing room floor, cool room floor, storage area floor, truck floor), 20 sponge swabs of surfaces including egg contact surfaces (conveyor - from shed; into the machine; out from the machine, rollers – prior and post candling, suction cups, collection bucket/tray/trolley and wheels, waste bucket/tray, sorting table) and non-egg contact surfaces (pallets, milk crates, shelves, hand wash area – basin; spout; tap, wash up area – basin; spout; tap, door handles – cool room, walls, window sills, fans, cleaning equipment – brooms; sponges; squeegee) and, three dozen eggs. Additional samples were also taken where warranted, depending on the specific layout of the farms and grading facilities sampled.

A total of 17 premises were recorded as an Infected Premise (IP) in NSW following the start of the SE outbreak (Table 6). Microbiological testing resulted in SE isolations from a variety of sources on infected properties, including on farm (barn, cage and free-range areas), grading facilities, loading docks, car parks, offices (chairs) and staff lunchrooms (benches). On farm, specific areas where SE was detected included barn dirt floors and walls, laying huts, nest boxes, cages, chicken runs, feed and drinking stations, water puddles and creek water in free range areas, bait stations, manure (chicken, rat, mouse, cockroach and cow), shed walls and doors, fans, cleaning implements, metal buckets, milk crates, external shed feed silos, pathways and farm tractor wheels. At grading facilities, specific areas where SE was detected included candling conveyors, candler lights, sorting tables and rollers, packing aisles, egg washing containers, egg waste buckets, window ledges, door handles, cool rooms and chest freezers (inside and outside surfaces).

The 17 IPs were located in Colo Vale, Greater Sydney, Mangrove Mountain and Port Stephens. All of the properties had links to each other through transfer of materials or people (Table 6). The number of other poultry properties (including other IPs) that each of the 17 premises had direct links to, ranged from just one other poultry property to up to 15 (Table 6). Sixteen of the IPs had poultry onsite and one premise is a grading facility only (IP 7). Of the 16 IPs with poultry onsite, all were small to medium-sized operations and all had registered flocks apart from one IP (IP 6). The 16 IPs with poultry onsite had different production systems in place; 6 (38%) were free range/barn style, 7 (44%) were caged, 1 (6%) was barn style and caged, 1 (6%) was free range and caged and 1 (6%) had roosters only. Of the 16 IPs that had poultry onsite, six IPs (38%) had onsite egg grading facilities and 10 IPs (62%) had eggs graded off-site. Of the 10 IPs that had eggs graded off-site, 3 IPs (IP 113, IP 23, IP 111) moved unstamped/ungraded eggs to other grading facilities (Table 6). Figure 2 is provided as a visual accompaniment to Table 6. Figure 2 presents additional information to Table 6, including the direction of movement of materials between poultry properties and information on Dangerous Contact Premises (DCPs) and Trace Premises (TPs).

Table 6*:* Infected properties in NSW during the SE outbreak

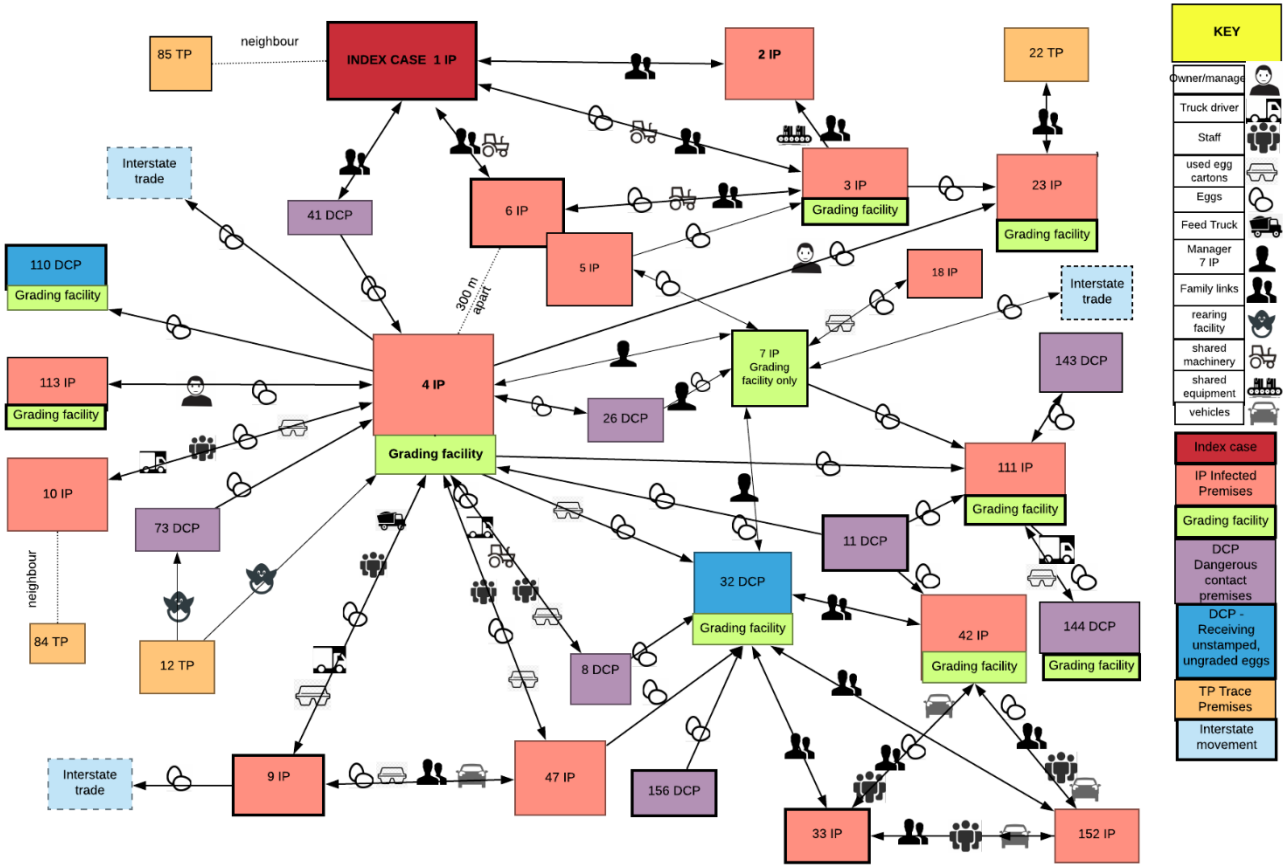
Infected Property (IP)	Date IP identified as SE-infected	Onsite grading facility	Number of direct links to other poultry properties (including IPs and description of movement between IPs) [a]
IP 1 (index case)	10/9/2018	No	Direct links: 4 (including 3 IPs) Movement between IP 1 and other IPs: IP 6: Machinery and family links; IP 3: Machinery, eggs and family links; IP 2: Family links *Note: IP 1 neighbours another poultry property*
IP 4 **Eggs traded interstate**	9/1/2019	Yes	Direct links: 15 (including 7 IPs) Movement between IP 4 and other IPs: IP 7: Manager; IP 133: Owner/manager and eggs; IP 10: Staff, truck driver, eggs and used egg cartons; IP 9: Eggs, used egg cartons, staff, truck driver and feed truck; IP 47: Staff, eggs and used egg cartons; IP 111: Owner/manager and eggs; IP 23: Owner/manager and eggs *Note: IP 4 neighbours IP 6*
IP 3	23/1/2019	Yes	Direct links: 5 (including 5 IPs) Movement between IP 3 and other IPs: IP 1: Family links; IP 2: Equipment and staff; IP 6: Machinery, eggs and family links; IP 3: Eggs; IP 5: Eggs
IP 6 **UNREGISTERED FLOCK**	25/1/2019	No	Direct links: 2 (IP 1 and IP 3) Movement between IP 6 and other IPs: IP 1: Machinery and family links; IP 3: Machinery, eggs and family links *Note: IP 6 neighbours IP 4*
IP 10	18/3/2019	No	Direct links: 1 (IP 4) Movement between IP 10 and IP 4: Staff, truck driver, eggs and used egg cartons *Note: IP 10 neighbours another poultry property*

Infected Property (IP)	Date IP identified as SE-infected	Onsite grading facility	Number of direct links to other poultry properties (including IPs and description of movement between IPs) [a]
IP 113	11/4/2019	Yes	Direct links: 1 (IP 4) Movement between IP 113 and IP 4: Owner/manager and eggs *Note: Movement of unstamped/ungraded eggs to other grading facilities*
IP 23	17/4/2019	Yes	Direct links: 3 (including 2 IPs) Movement between IP 23 and other IPs: IP 3: Eggs; IP 4: Owner/manager and eggs *Note: Movement of unstamped/ungraded eggs to other grading facilities*
IP 111	7/5/2019	Yes	Direct links: 5 (including 2 IPs) Movement between IP 111 and other IPs: IP 7: Eggs; IP 4: Eggs *Note: Movement of unstamped/ungraded eggs to other grading facilities*
IP 9 **Eggs traded interstate**	10/5/2019	No	Direct links: 2 (IP 4 and IP 47) Movement between IP 9 and other IPs: IP 4: Eggs, used egg cartons, staff, truck driver and feed truck; IP 47: Eggs, used egg cartons, vehicles and family links
IP 47	23/5/2019	No	Direct links: 3 (including 2 IPs) Movement between IP 47 and other IPs: IP 9: Eggs, used egg cartons, vehicles and family links; IP 4: Staff, eggs and used egg cartons

Infected Property (IP)	Date IP identified as SE-infected	Onsite grading facility	Number of direct links to other poultry properties (including IPs and description of movement between IPs) [a]
IP 7 **Eggs traded interstate**	31/5/2019	*Grading facility only*	Direct links: 6 (including 4 IPs) Movement between IP 7 and other IPs: IP 5: Eggs; IP 4: Manager; IP 111: Eggs; IP 18: Eggs and used egg cartons
IP 5	14/6/2019	No	Direct links: 2 (IP 3 and IP 7) Movement between IP 5 and other IPs: IP 3: Eggs; IP 7: Eggs
IP 18	9/7/2019	No	Direct links:1 (IP 7) Movement between IP 18 and IP 7: Eggs and used egg cartons
IP 2	20/11/2019	No	Direct links: 2 (IP 1 and IP 3) Movement between IP 2 and other IPs: IP 1: Staff; IP 3: Equipment and staff
IP 152	2/3/2020	No	Direct links: 3 (including 2 IPs) Movement between IP 152 and other IPs: IP 42: Staff, eggs, vehicles, family links; IP 33: Staff, vehicles, family links
IP 42	11/3/2020	Yes	Direct links: 4 (including 2 IPs) Movement between IP 42 and other IPs: IP 33: Staff, eggs, vehicles, family links; IP 152: Staff, eggs, vehicles, family links
IP 33	12/3/2020	No	Direct links: 3 (including 2 IPs) Movement between IP 33 and other IPs: IP 42: Staff, eggs, vehicles, family links; IP 152: Staff, vehicles, family links

[a] Direct links refer to the movement between poultry premises of eggs, equipment, humans (*e.g.* staff), vehicles, equipment etc.

Figure 2: NSW SE outbreak network diagram



2.3.3 National Surveys

Detection of *Salmonella* in samples from environmental testing of pooled faeces and dust in the egg production environment has been shown to strongly correlate with the within-layer flock prevalence of *Salmonella* and forms the basis of most monitoring programs for *Salmonella* in the poultry industry internationally (for a review see (J. J. Carrique-Mas & Davies, 2008)). Egg collection and packing areas are also important potential reservoirs for external contamination of eggshells. In addition, as *Salmonella* shedding by poultry is variable, a number of longitudinal studies have been undertaken in an effort to gain further insight (Gole, Torok, Sexton, Caraguel, & Chousalkar, 2014).

2.3.3.1 NSW Food Authority egg farm and egg grading facility surveillance program

A surveillance program for egg farms and egg grading facilities was introduced in July 2013. The aim of the program is to gather information on the prevalence of *Salmonella* on these premises. Table 6 and Table 7 display a summary of the *Salmonella* prevalence data and the *Salmonella* serotypes detected in *Salmonella*-positive samples, respectively. Due to the limited number of businesses surveyed each year, conclusions drawn from any observed trends in the data must be treated with caution. The percentage of egg businesses surveyed annually varies, as does the percentage of businesses surveyed which had a *Salmonella* detection. The majority of egg businesses surveyed from July 2013 to June 2018 (62%; 33/53) were positive for *Salmonella*. This is a higher prevalence than previously reported in an egg farm survey conducted by the NSW Food Authority between December 2010 and November 2011 (NSW Food Authority, 2013a), in which just under half (45%; 22/49) of all egg businesses surveyed were positive for *Salmonella*. From 2013 to 2018, a total of 21 serovars were identified from the group of isolates selected for serotyping. The only *Salmonella* serotypes detected at all businesses surveyed were ST and SI. *S*. Bareilly was the most common serotype detected in July 2013 - June 2014 and July 2015 - June 2016. While SI was the most common serotype isolated in July 2014 - June 2015, July 2016 - June 2017 and July 2017 - June 2018 and, the second most common serotype isolated in July 2015 - June 2016. ST was the second most common serotype isolated, either alone or alongside other serotypes, in July 2013 - June 2014, July 2014 - June 2015 and July 2016 - June 2017. ST and SI have both been linked to egg related outbreaks in NSW (see Section 2.3.1), while S. Bareilly has only been linked to two foodborne outbreaks from 2013 to 2020 involving sushi (Communicable Diseases Branch, 2017, *In print-b*). In the egg farm survey conducted by the NSW Food Authority between December 2010 and November 2011 (NSW Food Authority, 2013a), multiple serovars (*n*=17) were also isolated across the *Salmonella*-positive egg farms in the survey. ST was the most prevalent serotype (30%; 39/130) on egg farms in the survey, followed by SI (19%; 25/130) and *S*. Senftenberg (14%; 18/130) (NSW Food Authority, 2013a).

Table 7*: Salmonella* prevalence data obtained during the NSW Food Authority egg farm and egg grading facility surveillance program

Sampling time period	Number of businesses surveyed	% of businesses surveyed which had a *Salmonella* detection	% of environmental samples in which *Salmonella* was detected[a]	% of egg samples in which *Salmonella* was detected[a]	% of isolates serotyped
July 2013 - June 2014	9	89% (8/9)	22% (42/192)	0 (0/40)	60% (25/42)
July 2014 - June 2015	8	63% (5/8)	21% (32/153)	0 (0/40)	28% (9/32)
July 2015 - June 2016	13	70% (9/13)	9% (32/374)	2% (1/59)	100% (33/33)

Sampling time period	Number of businesses surveyed	% of businesses surveyed which had a *Salmonella* detection	% of environmental samples in which *Salmonella* was detected[a]	% of egg samples in which *Salmonella* was detected[a]	% of isolates serotyped
				The positive egg sample was taken after grading.	
July 2016 - June 2017	17	35% (6/17)	12% (41/345)	9% (2/23) Both positive egg samples were eggs taken directly from the laying shed prior to washing.	98% (42/43)
July 2017 - June 2018	6	83% (5/6)	13% (22/167)	0% (0/27)	100% (22/22)

[a]Environmental samples consisted of boot swabs, stock feed and faecal samples and each egg sample consisted of between six and twelve pooled eggs.

Table 8: *Salmonella* serotype data obtained during the NSW Food Authority egg farm and egg grading facility surveillance program

Salmonella serotype	Number of serotype detections and percentage (%) of the total number of isolates serotyped				
	July 2013 - June 2014	July 2014 - June 2015	July 2015 - June 2016	July 2016 - June 2017	July 2017 - June 2018
S. Abortusovis			2 (7%)		
S. Adelaide				2 (5%)	
S. Agona			4 (12%)	1 (2%)	2 (10%)
S. Bareilly	9 (36%)		13 (39%)	1 (2%)	
S. Bareilly / Typhimurium	1 (4%)				
S. Bovismorbificans				1 (2%)	

Salmonella serotype	Number of serotype detections and percentage (%) of the total number of isolates serotyped				
	July 2013 - June 2014	July 2014 - June 2015	July 2015 - June 2016	July 2016 - June 2017	July 2017 - June 2018
S. Infantis	2 (8%)	4 (44%)	7 (21%)	10 (24%)	5 (23%)
S. Liverpool				13 (4%)	
S. Livingstone		1 (11%)			
S. Mbandaka		1 (11%)			1 (5%)
S. Montevideo	2 (8%)				
S. Ohio	1 (4%)		1 (3%)		1 (5%)
S. Orion			2 (7%)		
S. Seftenberg			1 (3%)	1 (2%)	
S. Singapore	1 (4%)				1 (5%)
S. subs 1		1 (11%)		5 (12%)	3 (14%)
S. subs 1 ser 4,5:-:- (non motile)	1 (4%)				
S. Tennessee		1 (11%)			
S. Typhimurium	8 (32%)	1 (11%)	3 (10%)	5 (12%)	9 (5%)
S. Virchow				1 (2%)	
S. Worthington				2 (5%)	

2.3.3.2 National Surveys

In QLD, two microbiological surveys of egg farms were undertaken between October and November 2014 (Cuttell et al., 2014) and between May and October 2015 (Safe Food Production Queensland, 2015). The surveys were designed to replicate the survey of egg farms conducted by the NSW Food Authority between December 2010 and November 2011 (NSW Food Authority, 2013a), in order to enable comparison of cross-jurisdictional data. However, caution should be taken when comparing the studies, as the three surveys sampled farms at one point in time only. As already noted, *Salmonella* prevalence may vary over time on individual farms and from farm to farm. The NSW survey

covered 49 farms (20 egg producers, 29 egg producers/graders), representing 25% of the total number of egg businesses licensed at the time of the survey. Of the 49 egg farms included in the NSW study, 30 (61%) used a free-range system, 15 (31%) used a cage-based system and four (8%) used a barn system. The production volume from farms included in the NSW survey ranged from <1,000 eggs per day up to 250,000 eggs per day.

The 2014 QLD survey (Cuttell et al., 2014) covered 21 farms, representing approximately 25% of egg-producing businesses accredited with Safe Food Production Queensland (SFPQ) and more than 75% of the state's egg production volume (~2.5 million eggs/day). Of the 21 farms, 72% (15/21) were egg producers and 28% (6/21) were processors. Of the 21 farms included in the study, 9 (43%) used a caged system, 10 (47.5%) used a free-range system (of which 4 were free-range organic) and 2 (9.5%) used a barn system. In farms with mixed production types, the production type that comprised the majority of the farm's commercial activities was sampled. For barn and free-range systems, faecal material samples and boot swabs were collected from laying sheds. For caged systems, faecal material samples, as well as sponge swabs of belts, cages or floors, were collected. From each farm, samples were collected from 1, 2 or 4 layer sheds depending on the farm's production size. Of the 21 farms surveyed, 38% (8/21) were categorised as small (flock size ≤ 15,000), 29% (6/21) were categorised as medium (flock size ≤ 50,000) and 33% (7/21) were categorised as large (flock size > 50,000). From each shed, two samples were collected. Stock feed and water samples were collected from bulk reservoirs, which differed from the NSW Food Authority in 2010/2011 survey (NSW Food Authority, 2013a) where feed and water were sampled at the point of consumption. Eggs were excluded from the survey, due to the number of egg samples required to generate statistically significant results. More than 100 environmental (pooled faecal material and boot/sponge swabs) and 42 farm input (bulk feed and water) samples were collected (total number of samples 148). A farm was classified as 'positive' if any sample from that farm was positive for *Salmonella*. A shed was classified as 'positive' if either the faecal material or boot/sponge swab was *Salmonella*-positive. Farms, sheds and inputs were classified as 'negative' if all relevant samples were negative. However, the authors of the report emphasised that due to the limited number of samples collected, negative results were not a guarantee of the absence of *Salmonella* on the farm. Furthermore, variability in factors that influence the rate of *Salmonella* shedding in infected birds may also lead to false negative results.

For each *Salmonella*-positive sample, one isolate was recovered and subjected to serotyping. While further data analysis was undertaken to consider *Salmonella* prevalence by production system type, flock size and flock age at the shed and farm level, the authors of the report note that the level of sampling undertaken for the survey means that statistically valid conclusions could not be made on any observed differences. *Salmonella* was detected on 57% (12/21) of the QLD egg farms. ST was detected on 14% (3/21) of farms and no farm was positive for SE. There were no *Salmonella* detections in bulk feed or water samples. The authors state that these results were not unexpected, given that 95% (20/21) of farms surveyed used town supplied or bore water which are relatively low risk water supplies for microbiological contamination (compared to surface water) and, 66% (14/21) of farms purchased feed from an accredited supplier. Thirty-five isolates were serotyped from single isolates collected from 35 positive samples. In total, fifteen serovars were isolated and ST was the most common serovar (20%; 7/35) isolated, followed by *S*. Agona and SI (both 12%; 4/35).

The 2015 QLD survey (Safe Food Production Queensland, 2015) was conducted over a longer sampling period (6 months) than the 2014 survey (2 months) and this may have had temporal effects on the *Salmonella* detection rate compared to 2014. The 2015 survey covered approximately 63% of egg-producing businesses accredited with SFPQ. The list of participants for the 2015 survey excluded farms that had already been sampled during the 2014 survey. In total, 27 participants were chosen, consisting of 25 egg producers, one producer operating under a PSA[3] and one

[3] A PSA allows approved egg producers to supply eggs exclusively to an egg processor. A producer who is operating under a PSA cannot sell eggs directly to the public, or to anyone else other than the egg processor identified in their accreditation application.

processor. As in 2014, different types of egg production systems were sampled in 2015. In the 2015 survey, a greater proportion of small, free range systems were sampled than in the previous year. Of the 27 sampled farms, 22 (81%) were free range and 5 (19%) were caged. No barn systems remained to be sampled from 2014. In the 2015 survey, a greater proportion of small, free range systems were sampled than in the previous year. Regarding flock size, sampled farms ranged from < 1,500 birds to > 40,000 birds. The majority of sampled farms (70%; 19/27) were categorised as "lifestyle" with a flock size of less than 1,500 birds. Of the remaining farms, 19% (5/27) were categorised as small (1,600 – 15,000), 4% (1/27) were categorised as medium (15,001 – 40,000) and 7% (2/27) were categorised as large (>40,001). The sample collection and analysis methodology was essentially the same as in 2014 except feed and water samples were not included in the 2015 survey. Ninety environmental samples from 27 egg farms were collected for *Salmonella* testing.

Salmonella was detected across both types of production systems (i.e. free range or cage). For each *Salmonella*-positive sample, 8 isolates were recovered and subjected to serotyping. This method was different from the 2014 survey where only 1 isolate was selected for serotyping. Just over half of the QLD egg farms surveyed in 2015 were *Salmonella*-positive (55%; 15/27). In the 2015 survey a greater number (33%; 9/27) of ST-positive farms identified than in the previous surveys (Cuttell et al., 2014; NSW Food Authority, 2013a). Of note, one North QLD free range farm was positive for SE (PT26 and Reaction Does Not Conform (RDNC)) from environmental samples. Further confirmatory testing was undertaken by Biosecurity QLD and all results were negative. SE PT26 has previously been isolated from a limited number of samples from cattle and crocodiles and commercial broilers, all from the Atherton Tablelands. PT26 does not appear to be closely associated with poultry and investigations to date have indicated little potential for systemic colonisation of layers.

In total, 49 isolates were typed from 35 *Salmonella*-positive samples. ST was the most common serovar when the results were examined at the farm-level (9/27 farms; 33%). *S*. Anatum was the second most common type in samples reported in 2015 compared to SI in 2014. While in the NSW survey (NSW Food Authority, 2013a), ST was the most prevalent serotype accounting for 30% (39/130) of all the *Salmonella* positive samples, followed by SI (19%; 25/130). In comparing the results of the 2014 and 2015 QLD surveys and the NSW 2010/2011 survey, *Salmonella* was widespread on QLD egg farms (2014; 57%; 2015; 55%) and the results were comparable to NSW results from 2010/2011 (45%; 22/49). In the QLD surveys, ST was found on 14% (2014) and 33% (2015) of farms, compared with 20% (10/49) of NSW farms. The authors proposed that the higher prevalence of ST in the 2015 QLD survey (Safe Food Production Queensland, 2015) was likely due to the increased sensitivity of the laboratory testing, in that more *Salmonella* isolates were typed from each sample. It was predicted that the results of the 2015 survey are likely a more accurate representation of the ST prevalence in QLD (Safe Food Production Queensland, 2015).

In WA, Sodagari et al. (2020) investigated the occurrence and distribution of *Salmonella* in commercial layer farming environments of 26 flocks belonging to seven egg businesses (3 free-range, 3 barn-laid and 1 with both production systems) (Sodagari et al., 2020). Between November 2017 and June 2018, a total of 265 environmental samples of dust, feed, water, pooled faeces, and boot swabs were tested for detection of *Salmonella* according to standard culture-based methods. Dust (53.8%; 28/52) and pooled faecal (54.5%; 18/33) samples provided the highest *Salmonella* recovery rates. *Salmonella* was isolated from the environments of all seven egg businesses and from 23 of the 26 (88.5%) sampled flocks. *Salmonella* was recovered from all (11/11) of the free-range flocks as well as from 80% (12/15) of the barn-laid flocks. Nine serovars were identified from 93 *Salmonella* culture-positive environmental samples. ST (64.5%; 60/93) and SI (22.5%; 21/93) were by far the most prevalent serovars. Although ST was the only detected serovar in the environmental samples collected from two egg businesses (1 free-range and 1 barn-laid), there was more than one serovar detected in most of the other egg businesses. ST and SI co-existed in flock houses sampled from two of the free-range egg businesses. Moreover, two of the barn-laid egg businesses harboured four

and three different serovars in their flocks. Thirty-three ST isolates recovered from different flocks were further subtyped using MLST. All 33 ST isolates were characterized as ST-19.

Gole et al. (2014) undertook a longitudinal survey on two known ST-contaminated commercial layer farms within Australia, both with multiaged flocks housed in the same shed (Gole, Torok, et al., 2014). In order to select cages for the longitudinal study, a cross-sectional survey was conducted of two commercial layer cage sheds from two different farms with a history of *Salmonella* infection. The study sheds, shed A (from farm A) and shed B (from farm B), included multiaged flocks, with each age class housed in separate rows. A single age class flock was selected for sampling in each shed. In shed A, the selected flock included 1,320 cages of 32-week-old birds (5 birds per cage, for an approximate total of 6,600 birds), while the selected flock in shed B included 1,300 cages of 34-week-old birds (5 birds per cage, for an approximate total of 6,500 birds). To ensure that at least several cages positive for *Salmonella* would be identified, 78 cages per flock were sampled. The initial prevalence of *Salmonella* (based on faecal sampling, *n*=78) in flocks A and B were 26.9% and 39.7%, respectively. Cages selected for the longitudinal study, were five *Salmonell*a-positive cages each from farm A (3 cages of ST PT9, 1 cage each of SI and *S*. Orion) and farm B (2 cages of ST PT9, 1 cage each of SI, *S*. Agona and *S*. Oranienburg), as well as two *Salmonella*-negative cages per farm. Cages positive with different *Salmonella* serovars were selected in order to enable the dynamics of *Salmonella* shedding of various serovars to be investigated over a prolonged period of time. Over the 40 weeks of sampling, the highest prevalence of *Salmonella* was detected in dust (42%), followed by in egg belt (28%), faecal (20%) and eggshell (4%) samples. The prevalence of *Salmonella* in both flocks A and B increased during the later stages of lay. Gole et al. (2014) proposed that stress induced by moulting[4] or the introduction of a new batch of birds within the shed may have resulted in higher shedding of *Salmonella* in the environment, but that further controlled studies are required. Serotyping results confirmed that *S*. Oranienburg was the most frequently (76.92%) reported serovar, followed by ST PT9 (11.54%), *S*. Worthington (8.46%), *S*. Agona (3.08%), *Salmonella* subsp. 1 serotype 4,5,12:-:- (1.54%), and *Salmonella* subsp. 1 serotype rough g,s,t:- (0.77%). Out of all eggs tested, 7.17% (19/265) of the eggshells were *Salmonella* positive in flock B and one eggshell out of 256 (0.39%) was reported as *Salmonella* positive in flock A. Of the 20 *Salmonella*-positive eggshell samples, 18 were positive for *S*. Oranienburg, one was positive for *S*. Worthington and one was positive for ST. In Australia, there have been no reported foodborne outbreaks involving *S*. Oranienburg and eggs. However, an outbreak of *S*. Oranienburg linked to eggs occurred in 2016 in the USA (CDC, 2016; FDA, 2016).

2.3.3.3 International Surveys

In NZ, a cross-sectional survey was conducted between October and December 2016, to investigate the prevalence and serotypes of *Salmonella* in the feed, laying sheds (faeces, dust and boot or manure belt swabs), and packhouses (egg contact surfaces) of commercial egg layer farms (Kingsbury, Thom, Erskine, Olsen, & Soboleva, 2019). The surveyed farms represented 20% of the total egg producers and contained 46% of total laying hens in NZ (1.60 million of 3.48 million), ranging from 500 to ~400,000 laying hens per farm. The survey aimed to achieve approximately 50% each of farms defined as high production farms (flock size > 20,000), and small production farms (flock size ≤ 20,000). The total number (67) of sheds sampled contained 25% (0.87 million of 3.48 million) of all commercial laying birds in NZ. Sheds surveyed included 16 conventional cage, 9 colony cage, 34 free-range and 8 barn laying sheds. Of the 28 farms sampled, *Salmonella* was detected on 12 of 28 surveyed farms (42.9%). Of the *Salmonella* positive farms, 12 (42.9%) had at least one *Salmonella*-positive sample and a third (4/12) had only one positive sample. Of the 43 (13.3%) of 323 *Salmonella*-positive samples, dust samples had the highest prevalence (28.4%; 19/67), followed by boot or manure belt swabs (16.4%; 11/67), faeces (10.4%; 7/67), packhouse egg contact surfaces (5.7%; 5/87) and feed (3.0%; 1/33). One *Salmonella* isolate per positive sample was selected (43 in total) for further characterisation.

[4] Moulting means a life stage during which hens stop laying eggs and shed their feathers.

Salmonella-positive packhouse samples were only identified on the three farms in which the highest number of positive laying shed samples were obtained, and isolates were genetically related (as determined by single nucleotide polymorphism analyses) suggesting cross-contamination between the laying shed and packhouse surfaces. *Salmonella* strains isolated from the layer farm environment grouped into five serotypes on the basis of genoserotyping outputs. The most common serotype was SI (19 isolations), followed by *S*. Thompson (15 isolations), ST (6 isolations), *S*. Anatum (2 isolations) and *S*. Mbandaka (isolated once). Importantly, SE was not isolated. The five serotypes found in the survey have been isolated from both the NZ clinical and environmental settings at varying levels in recent years (2015 to 2018) (Kingsbury et al., 2019). All surveyed flocks were vaccinated and the six ST isolates from the survey all arose from flocks that had been vaccinated with the live, attenuated AviPro® Megan® Vac 1 vaccine. AviPro® Megan® Vac 1 aids in the reduction of ST (serogroup B), SE (serogroup D1) and *S*. Heidelberg (serogroup B). The majority of isolates in the survey belonged to different serogroups. SI, *S*. Thompson and *S*. Mbandaka belong to serogroup C1. *S*. Anatum belongs to serogroup E1. The authors proposed that the vaccine-mediated reductions in the prevalence of ST may result in a higher prevalence of *S*. Thompson and SI, due to these serotypes taking over and persisting in the ecological niche in place of ST. The authors note that this has also been proposed to attribute for increasing prevalence of SI in Europe and propose that to provide comprehensive coverage against *Salmonella*, serogroup C should be considered when investigating future vaccination regimens in NZ. The authors also report that the observed prevalence was lower compared with similar cross-sectional surveys carried out on Australian egg layer environments, likely due to differences in various on-farm interventions (including vaccination type), climate and endemic fauna.

2.3.4 Recalls of egg and egg products

Analysis of consumer level recalls provides some information on the foods and safety hazards that do or could enter the domestic food supply and pose a health risk. Foods may be recalled due to issues associated with contamination (*e.g.* microbial, biological toxins, chemical, foreign matter), non-compliant labelling, undeclared allergens, faulty packaging and for a variety of other reasons (*e.g.* unsafe levels of additives) (FSANZ, 2018). Information on consumer level recalls of egg and egg products in Australian States and Territories can be accessed on the FSANZ website (FSANZ, 2021b). At the time of writing, records were accessible for consumer level recalls that occurred from 17/10/2015 onwards. There was a total of nine consumer level recalls of egg and egg products between 17/10/2015 and 31/12/2020 (Table 9). This included seven recalls due to microbial contamination and two recalls involving inadequate egg cleaning and the potential for unacceptable dirty eggs. All seven recalls involving microbial contamination were a result of the detection of SE, with 6 consumer level recalls of eggs from implicated properties in NSW and one consumer level recall in VIC.

Table 9: Consumer level recalls of egg and egg products in Australia from 17/10/2015 to 31/12/2020[a]

Date	Location	Product	Outlet type	Reason
14/6/2019	NSW	The Egg Basket eggs	The Egg Basket and Flemington Markets	Potential microbial (*Salmonella* Enteritidis) contamination
7/5/2019	NSW	Port Stephens Eggs	Supermarkets and retailers	Potential microbial (*Salmonella* Enteritidis) contamination
16/4/2019	NSW	Steve's Farm Fresh Eggs	Retailers	Potential microbial (*Salmonella* Enteritidis) contamination

Date	Location	Product	Outlet type	Reason
6/4/2019	NSW and ACT	Southern Highland Organic Eggs	Supermarkets and retailers	Potential microbial (*Salmonella* Enteritidis) contamination
27/3/2019	NSW	Ash and Son Eggs	Supermarkets and retailers	Potential microbial (*Salmonella* Enteritidis) contamination
20/3/2019	ACT, NSW, VIC, TAS and SA	Bridgewater Poultry Eggs	Supermarkets and retailers	Potential microbial (*Salmonella* Enteritidis) contamination
7/9/2018	NSW	Glendenning Farms eggs	Supermarkets and retailers	Potential microbial (*Salmonella*) contamination
17/5/2018	QLD	Sunny Queen Organic Free Range	Supermarkets	Some eggs may not have been cleaned to the usual required standard
22/01/2016	NSW and QLD	Ovaston Organics Eggs		System failure: potential for unacceptable dirty eggs

[a] Data accessed from the FSANZ website (FSANZ, 2021b).

2.4 Risk characterisation

Rates of salmonellosis are higher in Australia than other developed nations, including the USA and Europe (Laura Ford et al., 2016). *Salmonella* is the second highest cause of bacterial enteric illnesses in Australia after *Campylobacter* (Communicable Diseases Branch, *In print-b*). ST is the most common *Salmonella* serovar linked to foodborne illness in Australia (Communicable Diseases Branch, *In print-b*). Domestic microbiological surveys have shown that ST was amongst the most prevalent serotypes on commercial egg farms in NSW (see Section 2.3.3.1), QLD (Cuttell et al., 2014; Safe Food Production Queensland, 2015) and WA (Sodagari et al., 2020). This in accordance with the frequent isolation of this serotype in egg-related human salmonellosis cases nationally. McClure et al. (2021) estimated that approximately half of all notified cases of non-typhoidal salmonellosis acquired in NSW during 2008–2019 were due to direct or indirect transmission from layer chickens. Foodborne illness reports in NSW from 2013 to 2020 (Communicable Diseases Branch, 2014, 2015, 2016, 2017, 2018, 2019, *In print-a*, *In print-b*) reported a total of 52 egg-related outbreaks, with ST (83%; 43/52) the most common serovar implicated.

The NSW Food Safety Strategy 2015–2021 included a specified 30% reduction target for foodborne salmonellosis from 2014 levels (NSW Food Authority, 2015b, 2015c). Data provided by NSW Health from January 2014 to July 2018 has shown that NSW has already achieved its 30% reduction target in foodborne salmonellosis (NSW Food Authority, 2019b). During this time, the number of cases of foodborne salmonellosis attributed to ST declined by 65%; from 2547 cases in 2014 to 893 cases in 2018 (Communicable Diseases Branch, 2016, *In print-b*). Prior to 2014, many reported foodborne outbreaks in NSW were linked to poor handling of eggs and hygiene at retail food service. Several measures were implemented to address this, including mandatory education and food safety training on the risks of raw egg use, improved cleaning and sanitising for retail businesses, guidelines for the safe use of eggs which made it an offence in NSW to prepare raw egg foods without adequate processing, and training and guidance for local council Environmental Health Officers to focus on areas of highest food safety risk during inspections. Tighter food safety

regulations for the handling of eggs and large scale vaccination of layer flocks against ST, are likely responsible for the proportion of *Salmonella* infections attributed to layers in NSW declining from approximately 50% in 2009-2011 to approximately 25% in 2017-2019 (McLure, Shadbolt, Desmarchelier, Kirk, & Glass, 2021). Of the 43 outbreaks from 2013 to 2020 caused by ST, 75% (32/43) were due to ingestion of "*raw*" (56%; 24/43) or "*undercooked*" (19%; 8/43) eggs or egg products. For the remaining ST outbreaks (*n* = 10), where further detail was provided, inadequate cleaning of equipment (*n* = 3) and cross-contamination (*n* = 1) were reported as contributing factors. Restaurants (47%; 20/43) were the most common outbreak setting, followed by bakeries (16%; 7/43) and take-away venues (12%; 5/43). This indicates that further work could be undertaken to educate and offer guidance materials to the retail and food service businesses and consumers, on risky egg handling practices (NSW Food Authority, 2020e), safe egg handling practices (NSW Food Authority, 2021c) and the safe preparation of raw egg products (NSW Food Authority, 2016a). Successful initiatives have already been undertaken by the NSW Food Authority in collaboration with local councils, to help retail businesses in the use of raw egg mayonnaise and to assist in increasing compliance and education in this sector (NSW Food Authority, 2020a).

The incidence of salmonellosis in NSW compared to the 5 year annual mean has continued to decline by a further 15% in 2019 (Communicable Diseases Branch, *In print-a*) and 23% in 2020 (Communicable Diseases Branch, *In print-b*). However, the number of cases of foodborne salmonellosis attributed to ST slightly increased in 2019 (901 cases) and 2020 (1120 cases) (Communicable Diseases Branch, 2016, *In print-b*). The slight increase in ST cases in 2018-19 was linked to eggs from a single egg farm, which was the source of approximately 20% of all ST cases in NSW (NSW Food Authority, 2019a). This farm's contribution to illness was exacerbated further down the supply chain by poor handling of eggs in some businesses, including failure to clean and sanitise surfaces or equipment, and the use of raw egg products (NSW Food Authority, 2019a). The farm implemented additional cleaning and sanitising of farm grading equipment and was following up with vaccination of birds to reduce ST (NSW Food Authority, 2019a). In 2020, one large outbreak was primarily responsible for the number of ST notifications increasing by 24% when compared to 2019 (Communicable Diseases Branch, *In print-b*). This ST outbreak occurred between January and April 2020 and affected over 1,000 people nationally, including over 200 in NSW (NSW Food Authority, 2020b). As part of the national effort to find the source of the outbreak, over 590 food and environmental samples were obtained by the NSW Food Authority (NSW Food Authority, 2020b). This included over 300 bagged salad items, which had the strongest epidemiological link to the outbreak (NSW Food Authority, 2020b). Despite an extensive investigation no source for the outbreak was found (NSW Food Authority, 2020b).

While ST was responsible for the vast majority of egg-related outbreaks reported from 2013 to 2020, of most concern is the emergence of illness due to locally acquired SE. Nationally SE was the causative agent in a total of 245 cases of foodborne illness linked to eggs from domestic commercial egg laying poultry flocks. Since the start of the SE outbreak in September 2018, 17 infected premises (IPs) have been identified within NSW, from Colo Vale, Greater Sydney, Mangrove Mountain and Port Stephens. Prior to September 2018, SE had not been detected in commercial egg laying poultry flocks in NSW. The SE outbreak strain was designated SE 7A and had not previously been reported or observed in Australia or elsewhere in the world (Quinteros et al., 2021). As all farms identified as SE positive had clear links to the first identified infected property, it was thought that SE 7A was likely introduced through human associated horizontal contacts and international travel (Quinteros et al., 2021). In support of this, all human cases of SE 7A were related to table egg consumption and the epidemiological picture would have been very different if another primary vector (*e.g.* wild birds or rodents) or food source was involved (Quinteros et al., 2021). The spread of SE in Australia is considered a significant public health and biosecurity concern. SE presents a higher risk to consumers and the Australian egg industry because it can colonise the internal contents of eggs during their development via infection of the oviduct of chickens. The systemic nature of infection of the Australian SE isolate (SE 7A) and its ability for ovarian colonisation, was confirmed in a laboratory-controlled exposure of laying hens to SE 7A (P. C. Scott et al., 2020).

In response to the risk that trans-ovarian strains of SE may become established in Australia, the Chair of the Food Regulation Standing Committee (FRSC) requested that FSANZ review Standard 4.2.5 of the Code to address the risk of SE to human health. When Standard 4.2.5 was developed in 2011, FSANZ considered ST as the main microbial hazard for eggs in Australia. The risk from SE was not specifically considered, as it was not seen to be an issue in Australia at the time. FSANZ reports that it is investigating if Standard 4.2.5 remains effective and current given the emergence of SE, with a focus on two areas of particular concern (FSANZ, 2021a). The first identified area of concern is the interface between biosecurity and food safety measures on-farm, including bird health and controls and monitoring for harmful bacteria (especially *Salmonella*). The second identified area is the adequacy of current requirements for the safe production of eggs and egg products, including monitoring of layer flocks for SE, temperature control for intact shell eggs in the supply chain, and traceability (*e.g.* egg stamping). Submissions to FSANZ on the review of Standard 4.2.5 were invited before the 8th November 2021, after which time it will be determined whether a proposal to amend the Code is necessary, or whether other measures should be considered to manage identified issues.

The following risk characterisation focuses on control measures to minimise the risks associated with SE. However, many recommended SE risk reduction practices are also applicable against other salmonellae as well as a broad spectrum of other poultry pathogens. Reducing the prevalence of SE infection in flocks of commercial laying hens is critical for reducing egg-transmitted human illness. The most promising strategy for SE control is the application of multiple interventions distributed throughout the egg production cycle. The following risk characterisation includes discussion of control measures already identified by FSANZ (2021a), including biosecurity, flock testing, temperature control for intact shell eggs and traceability. In addition, an overview of vaccination has been included, as it is one of the most prominent risk reduction practices specifically applicable to SE in chickens. The risk characterisation has focussed on studies in comparable high-income countries, but relevant studies from other global regions were included if relevant production systems, management and housing practices applied. In countries in which SE is endemic, comprehensive control programs incorporating a combination of broad-spectrum risk reduction practices plus vaccination and testing targeted to SE, have achieved documented success in reducing both the prevalence of infection in egg-laying flocks and the incidence of disease transmission to humans. However, it should be noted that the nature of public health interventions often means that evaluating their impact is complex as they are often implemented in combination and/or simultaneously (O'Brien, 2013).

2.4.1 Biosecurity

Biosecurity refers to those measures taken to prevent or control the introduction and spread of infectious agents to a flock. Effective biosecurity practices are a critical component of any integrated program to reduce the probability that any potential pathogen, not only SE, will enter, spread through or leave the egg-production facility.

The main reservoir of zoonotic *Salmonella* is the gastrointestinal tract of warm-blooded animals including food-producing animals. *Salmonella* can also be present in the intestinal tract of wild birds, reptiles and occasionally insects. Feedstuff, soil, bedding, litter and faecal matter are commonly identified as sources of *Salmonella* contamination on farms. As *Salmonella* colonises the gastrointestinal tract, the organisms are excreted in faeces from which they may be transmitted by insects and other animals to a large number of places. The critically important relationship between environmental reservoirs of SE and infections of egg-producing chickens has been widely documented (Dewaele et al., 2012). Environmental conditions can directly affect opportunities for SE introduction, transmission and persistence in laying flocks. Direct contact between hens, ingestion of contaminated feed or faeces, movement of personnel and equipment, and airborne circulation of contaminated dust and aerosols all facilitate rapid and extensive horizontal dissemination of SE throughout laying flocks.

As *Salmonella* can be highly persistent in both infected birds and diverse environmental reservoirs, effective disease control requires intervention at more than one step in the complex egg production cycle. Preventing the introduction of

Salmonella into poultry facilities requires measures such as the implementation of stringent biosecurity measures, stocking exclusively with replacement pullets that are demonstrably uninfected, controlling population levels of rodent and insect pests and securing farms against access by wildlife (Robert H. Davies & Breslin, 2003). The possibility of indirect horizontal transmission of infections to subsequent flocks via contaminated environmental sources, can be minimised by thorough cleaning and disinfection of laying houses between flocks (Juan J. Carrique-Mas, Marín, Breslin, McLaren, & Davies, 2009).

The microbiological sampling test results from the 17 IPs during the NSW SE outbreak, highlighted the need for improvements in the level of biosecurity within and between premises. Humans likely contributed to the spread of SE, with SE detections outside of production areas (i.e. offices and lunchrooms) and on surfaces indicating inadequate sanitising of hands (e.g. SE was detected on door handles) and footwear (e.g. SE was detected on floors of grading rooms, pathways and receiving rooms). Vehicles were also implicated in the potential spread of SE (*e.g.* SE detections on farm tractor wheels, car parks and loading dock floors), as were items commonly transferred between IPs (e.g. SE was detected in eggs and on the shelves on which egg packaging was held). The complex network of movement of people, vehicles and equipment between premises and the time lag between introduction of SE into a premise and its establishment and detection on farm, also reconfirmed the importance of accurate record keeping for traceability. Data from the NSW SE outbreak revealed an average lag time between introduction and establishment/detection of SE of 8-14 weeks, with a maximum of 5 months (Catherine Fraser, personal communication).

The microbiological sampling test results from the 17 IPs during the SE outbreak, also revealed SE isolations from bait stations and rat and mice faeces. However, there was no direct evidence to pinpoint rodents as the source of the outbreak or a mode of transmission between IPs (Catherine Fraser, personal communication). However, within an infected property mice and rats have been reported to play an established role in the maintenance of SE infection on layer farms (for a review see (Ducatelle & Van Immerseel, 2011)). Mice and rats can acquire *Salmonella* infection from various sources. They may get infected through contact with faeces from infected pullets or hens, or else from infected wild birds. While rodents may not necessarily introduce *Salmonella* into a layer farm, they amplify the problem if present. Wild mice excrete SE intermittently in their faeces at a concentration of up to 10^4 cfu per dropping, which is consumed by chickens when mixed in their feed or bedding (R. H. Davies & Wray, 1995). Unfortunately, layer farms attract rodents by providing them with shelter, a source of food and access to water. Control measures can be put in place to significantly reduce the attractiveness of a farm to rodents by removing potential food sources (*i.e.* spilled feed, broken eggs and other waste) and removing potential sites of shelter (*i.e.* clearing surrounding areas of vegetation, debris and disused structures or equipment). However, it is difficult to completely rodent-proof a layer farm, therefore sufficient rodent control with bait stations is vital.

The perpetuation of SE infection in laying flocks is commonly related to the presence of a contaminated house environment. Effective terminal cleaning and disinfection is regarded as a necessary step for the elimination of SE from the laying environment. The aim of cleaning and disinfection of poultry houses should be to eliminate contamination of the building and equipment by SE and by organic matter that can harbour SE following repopulation. Inadequate terminal cleaning and disinfection practices were observed on a number of IPs during the NSW SE outbreak investigation. As one example, IP 5 reported that the last time they had poultry onsite was in June 2018. Nine months after this time, IP 5 returned negative environmental SE test results on the 4th of March 2019. Sheds at IP 5 were restocked with layers on the 13th of March 2019. IP 5 returned negative environmental SE test results six times between the 23rd of March and the 15th of April in 2019. However, clinical signs and positive serology confirmed the presence of SE in laying flocks at IP 5 in June 2019. IP 5 had direct links to two previously identified IPs (IP 3 and IP 7) through the transfer of eggs. In addition, while IP 5 did not have poultry onsite for around 9 months prior to restocking, manure had been present. *Salmonella* has previously been reported to survive for up to 2.5 years in dried bird faeces (Morse & Duncan, 1974). SE has also been shown to persist for at least a year in depopulated poultry

houses (R. H. Davies & Wray, 1996). This highlights the importance of undertaking cleaning and disinfection of poultry houses immediately after depopulation. In addition, cleaning should be thorough, including spots that are difficult to reach, such as ventilation tunnels. The problem of sanitation and decontamination is particularly difficult in free-range systems, with reports that viable SE persisted in soil samples after 8 months of depopulating a free-range breeding chicken farm (Robert H. Davies & Breslin, 2003). During the NSW SE outbreak response, sanitation procedures on farms in which SE was detected, included the eradication of contaminated flocks, thorough cleaning and disinfection of contaminated housing and safe disposal of contaminated manure. Several IPs in NSW required multiple rounds of decontamination before their premise was cleared of SE. Similar observations have been made overseas regarding variability in the level of effectiveness of decontamination activities undertaken at laying houses contaminated with SE and ST (Juan J. Carrique-Mas et al., 2009; Wales, Breslin, & Davies, 2006).

A number of factors need to be considered prior to undertaking decontamination activities. There is a wide range of disinfectant formulations available on the market and they have been reported to differ markedly in their efficacy. In addition, the efficacy of a disinfectant in the field is highly dependent on the level of residual organic matter remaining. In a study by Wales et al. (2006), 12 SE-contaminated caged layer houses were sampled prior to depopulation and once following depopulation, cleaning and disinfection. A variety of standard cleaning and disinfection procedures were employed at each of the 12 premises examined and none achieved elimination of SE. However, when the methods of cleaning and disinfection were compared, the greatest decreases in SE contamination were associated with initial dry cleaning, moderate to low residual organic material and, the use of aldehydes (formaldehyde or formaldehyde/glutaraldehyde combinations) for disinfection and/or fogging. The authors also reported the isolation of SE from the faeces of various wildlife vectors (including mice and rats) at numerous premises post-cleaning and disinfection. In a similar study, Carrique-Mas et al. (2009) investigated the effectiveness of various decontamination activities on 60 commercial laying houses that had housed laying flocks infected with SE or ST that were representative of all production systems (cage, barn, free-range). Intensive sampling was undertaken immediately after cleaning and disinfection as well as in the follow-on flock. The procedures investigated were: (1) a compound disinfectant consisting of a mixture of formaldehyde, glutaraldehyde and quaternary ammonium applied at the recommended concentration; (2) a 10% (vol/vol) dilution of the standard 37% commercial formalin, applied by a contractor; and (3) other disinfection procedures selected and applied by the farmer. The recovery of *Salmonella* in the cleaned and disinfected houses was variable, with samples from floor and dropping boards/belts (cage houses) and scratching areas (non-cage houses) being the most likely to remain contaminated. The best results were achieved using 10% formalin (fogging) applied by a specialist contractor, with a lower recovery of *Salmonella* from the treated surfaces in the house and, a lower rate of infected follow-on flocks and a reduction in the levels of infection in houses where an infected flock was present.

The second most effective agent was the blended disinfectant that also contained formaldehyde, in combination with glutaraldehyde and quaternary ammonium compounds. A number of other disinfection procedures were studied, however the authors noted that the diversity of treatments under study did not permit conclusions to be made regarding any particular product. The authors concluded that there appeared to be a widespread lack of knowledge amongst the farmers on the appropriate concentrations of disinfectants to be used. The confusion was proposed to be due to their reconstitution at a lower than optimal concentration, as is recommended for other uses. The authors stated that it would be preferable if a suitable concentration for disinfection of *Salmonella* in biofilms on soiled surfaces could be defined for all products, so that operators could obtain clear guidance on appropriate disinfection. It is also important to note that the study found that in 58% of cases where a negative post-cleaning and disinfection result was obtained, that there was evidence of infection in the follow-on flock. The authors propose that this is likely to be a consequence of the presence of other major sources of infection to the flocks, notably rodents.

The *Salmonella* Enteritidis Operational Response Plan includes an overview of farm clean-up, wash down and disinfection practices (Australian Eggs Limited, 2020b). The overview summarises the various chemical disinfectants commercially available and their advantages and disadvantages. The plan emphasises the added complexity of eradication of SE from a site, due to the probable concurrent contamination of passive carrier hosts (*e.g.* mice and rats, foxes, wild birds, flies, litter beetles, avian ectoparasites, environmental invertebrates, and domestic livestock and pets). The plan states that the likelihood of failure to eradicate SE from a site should be considered as a significant possibility and that a risk assessment should be undertaken prior to repopulating the farm.

Following the detection of SE in commercial NSW layer flocks, the Biosecurity (*Salmonella* Enteritidis) Control Order 2019 came into effect. The Control Order establishes minimum biosecurity standards for the poultry and egg industry and makes them legally enforceable under the Biosecurity Act 2015. Licensed egg producers were also required to regularly test for SE, under a revised control order introduced in mid-2020. The Control Order in NSW is in effect until the 30th of June 2024. The Control Order applies to all persons in charge of commercial poultry facilities in NSW. Amongst those requirements for egg and poultry producers, is a requirement to clearly demarcate the production area so that strict biosecurity measures can be enacted for people and items entering and exiting the identified production area. All entrances to the production area that are available for use as an entrance must have a clearly visible sign which lets people know that they are entering a production area and that they must have permission to do so. Prior to entry, all persons entering the production area must be provided with a copy of the Control Order, or information on how to access it. When a person seeks permission to enter the production area, the person in charge is only to grant permission if the person agrees to comply with any measures put in place on the premises and production area to implement this Control Order. Other areas covered by the order include signage, control of persons entering premises, handwashing, disposal of dead birds, vermin control and record keeping. These requirements are briefly summarised below.

The Control Order lays out requirements relating to production area entrances:

- entry into any production area must be via an entry marked as the entrance to the production area (a designated entrance will have appropriate signage, footwear and hand sanitising facilities)

- equipment and materials for decontaminating footwear or ‘shed boots’ that are to be only worn within the production area

- hand sanitising facilities

- parking for vehicles entering the premises, but not the production area

- equipment and materials for washing of wheels, footsteps and wheel arches of vehicles before and after accessing the production area

The Control Order lays out requirements relating to vermin control:

- any dead birds on the premises must be stored and disposed of in a manner that prevents vermin and other animals accessing dead birds

- all poultry housing, egg packing facilities and grading facility buildings in the production area must be constructed and maintained to prevent the entry of wild birds and limit access of vermin

- a vermin control strategy, designed to control all vermin on the premises, must be documented, developed and implemented on the premises

The Control Order lays out requirements relating to written records and how long the records are to be kept:

- the name of all persons entering the production area and the date of entry (12 months)

- the date of all deliveries received into the production area, and of all vehicles that remove anything from the production area, the nature and contents of the delivery or thing being removed and either the number plate and or the name of the company or person responsible for the delivery or removal is to be made or obtained within 1 month of the date of the delivery (12 months)

- the numbers and dates of all poultry mortalities that occur within the production area (12 months)

- where all poultry entering the production area have come from, and where all poultry exiting the production area are being moved to is to be made, or obtained within 1 month of the poultry entering or exiting the production area (12 months)

The Control Order lays out requirements relating to sanitising materials before use for transporting or storing eggs (unless they are being used on the same premises or within the same network of premises):

- cardboard egg flats and cartons are to be new or heat sanitised

- plastic egg flats and fillers are to be new or disinfected

- pallets are to be new, or cleaned to remove all visible organic matter and prior to use, must be stored in a clean area, that is not a rodent habitat or potential rodent habitat

Requirements are also laid out for poultry suppliers, receivers and pullet rearing facilities. Poultry of numbers of 100 birds or greater must not be supplied to a person or business unless that person or business is licensed for poultry egg or meat production under the Food Act and has a Property Identification Code (PIC) or has a provisional license and a PIC. Records of all poultry transactions including the license number and PIC must be kept for 12 months. Pullet rearing facilities can continue to receive birds without a Food Act licence but they must have implemented the Control Order and have a PIC.

The Biosecurity & Food Safety Compliance and Integrity Systems unit was tasked to inspect all licensed egg farms in NSW to ensure compliance with the Biosecurity (*Salmonella* Enteritidis) Control Order (for a review see (NSW DPI, 2021a)). Initially this required 241 egg farms to be assessed twice for compliance. As a result of these inspections, it was reported that an initial low level of compliance was observed. However, these findings were expected due to the increased requirements of the Control Order. Areas pertaining to the *Biosecurity Act 2015* and *Food Act 2003* requirements, where a low compliance level was observed, are briefly summarised below.

Areas where low levels of compliance were observed regarding *Biosecurity Act 2015* requirements:

- accurate signage defining the production area and appropriate control measures

- sufficient rodent control with bait stations regularly replenished and documented

- record keeping with respect to deliveries and entry to premises and production areas

Areas where low levels of compliance were observed regarding *Food Act 2003* requirements:

- appropriate fixtures, fittings and equipment in processing areas

- hygiene, sanitation and cleanliness of premises and equipment

Where areas of non-compliance were identified, an IBD was issued. The IBD detailed all actions required for the licensee to comply with the Control Order. Subsequently, a second phase of Control Order inspections was conducted to target those farms previously issued with an IBD. This resulted in 140 farms being inspected. It was reported that some facilities received multiple inspections as they had not yet met the requirements of the Control Order. In cases where continued corrective actions were identified, the breaches were considered minor in nature but were listed on the reports to ensure licensees implemented the required corrective actions. A total of 440 inspections were

conducted, resulting in 202 IBDs and 20 sanctions. As of the 1st of July, 2021, all licensed egg farms in NSW were considered compliant with the Control Order. A total of 35 licensees left the industry during the program.

Prior to the inspections in NSW, two published reports had also highlighted that there was room for improvement in biosecurity practices performed on Australian commercial layer farms (A. B. Scott et al., 2018; Sodagari et al., 2020). Scott et al. (2018) undertook a study from June 2015 to February 2016, to investigate the level of adoption of biosecurity practices performed on Australian layer farms (> 1,000 birds) and farmer-perceived importance of these practices. On layer farms, the median number of chickens was greatest on cage layer farms (40,000), followed by free range (32,000) and barn (17,500). The median number of sheds for layer farms was two for both cage and barn layer farms and three for free range layer farms. On-farm interviews were conducted on 25 commercially operating free range layer farms and nine cage layer farms, nine barn layer farms in the Sydney basin bioregion[5] and South East QLD. The objective of the on-farm survey was to describe farm design, structure, wild animal exposure, contact with other farms and biosecurity practices among the farm types to inform understanding of variation between these farms that could impact on the introduction and spread of infectious agents (with a specific focus on avian influenza virus). Biosecurity manuals were present on the majority of barn layer farms (89%) and free range layer farms (88%), but only on half (50%) of the cage layer farms. Rodent control was common across all farm types, being performed on 96% of farms. Equipment sharing between sheds was performed on 78% of cage layer farms, 78% of barn layer farms and 92% of free range layer farms. There was little disinfection of this shared equipment across all farm types, with disinfection of equipment between sheds occurring at none of the cage layer farms, 14% of the barn layer farms and 9% of the free range layer farms. Foot baths were used on 25% of cage layer farms, 67% of barn layer farms and 76% of free range layer farms. Hand washing/sanitation facilities were present on 89% cage layer farms, 89% of the barn layer farms and 96% of the free range layer farms. Visitor recording book were present on 25% of cage layer farms, 56% of the barn layer farms and 80% of free range layer farms. Restricted contact between farms was recorded for 89% of cage layer farms, 100% of barn layer farms and 67% of free range layer farms. Disinfection of vehicles between farms was reported to occur for 63% of cage layer farms, 67% of barn layer farms and 48% of the free range layer farms. The percentage of farms that depopulated entire sheds in one day differed significantly by farm type and occurred most commonly in barn layer farms (89%) and free range layer farms (88%). Most cage layer farms (78%) had multi-aged sheds and depopulated birds of a single tier or row simultaneously; only two cage layer farms (22%) depopulated entire sheds in one day. This generally means sheds are rarely empty, enabling the persistence and circulation of pathogens (A. B. Scott et al., 2018). Performing thorough sanitisation of sheds after depopulation of entire sheds was also significantly different amongst the farm types, with most barn (89%) and free range (88%) layer farms performing this practice compared to only 33% of cage layer farms. In relation to turnaround times, described as the time period from when sheds are depopulated of birds to when new birds arrive, barn and free range layer farms had an average turn-around time of approximately 23 days. However, there was one barn and one free range layer farm with no turnaround times, introducing new birds the same day that the previous flock was removed. For cage layer farms, the average turnaround time of empty cages (including multi-aged sheds where birds are still present inside the sheds) was 10.5 days. However, the average turnaround time was 22.8 days when only considering the two cage farms that depopulate the entire shed in one day. Scott et al. (2018) concluded that the variation amongst the layer farms in the level of biosecurity, may be due in part to the high level of private ownership and the absence of a mandatory governing body to enforce adoption and compliance of biosecurity practices. Scott et al. (2018) reported that cage layer farms tended to rate lowest in the level of biosecurity amongst the farm types. In the farmer self-assessment of the biosecurity compliance rating of their farm, cage layer farmers also gave their farms the lowest average rating compared to the other farm types. Scott et al. (2018) concluded that this demonstrated some degree of

[5] The Sydney Basin bioregion extends from just north of Batemans Bay to Nelson Bay on the central coast, and almost as far west as Mudgee.

awareness of farm biosecurity amongst the cage farmers and an acknowledgment of lower compliance than best practice. Scott et al. (2018) noted that most cage layer farms visited in their survey were old, family-run farms that had been passed onto the next generation and this is a particular feature of farms located in the Sydney basin region in general. This was highlighted to be in contrast with barn and free range layer farms, which are relatively new due to the recent expansion of cage-free egg production. Scott et al. (2018) proposed that the structural features of older farms to some extent limit compliance with current best practice and that it is likely that farmers with more recently established farms seek technical services and support more frequently than farmers with long established farms. However, Scott et al. (2018) did not capture information within their survey on the age of the farms or the level of biosecurity training received by farmers and other staff members.

Similarly, Sodagari et al. (2020) reported variable levels of compliance with basic biosecurity measures as well as high-risk egg handling practices, in a survey of non-cage egg businesses in WA between November 2017 and June 2018. The survey involved seven egg businesses (26 flocks), of which three were free-range, three were barn-laid and one business had both production systems: two barn-laid flocks and two free-range flocks (Sodagari et al., 2020). All of the businesses had in-house quality assurance systems and almost all had adopted a written food safety management statement. A written/documented HACCP system was also present in most of the farms. Nevertheless, few farm managers and staff were reported to follow any kind of either formal or in-house training on aspects of HACCP, food safety principles or biosecurity. All business had a policy of restricted person's access (staff and verified visitors only). Only one business (14%) maintained vehicle wheel washing practices for visitors. Only one business (14%) required workers and visitors to change their clothes while walking between sheds. At each shed entry, five businesses (71%) maintained handwashing facilities (basin) and footbaths filled with sanitiser. Other areas observed as needing improvement included systematic cleaning and sanitisation of sheds between production cycles, poor maintenance of pest control stations and baits and, inadequate practices to restrict rodent infestations and access to feed and sheds. Some high-risk egg handling practices were also observed, with three of the seven (43%) egg businesses indicating that the storage period prior to grading was as long as 48 hours. Moreover, in four of the seven egg businesses (57%), pre-graded eggs were typically stored at ambient temperatures (rather than at or below 15°C, as recommended in the Australian Egg Industry guidelines). Finally, the sanitising frequency of egg handling equipment was variable in terms of frequency and procedure. Daily sanitisation of egg handling equipment occurred in four (57%) of the visited farms, while weekly (14%) and even monthly (14%) sanitisation was reported by the remainder.

It is worth noting that recently in NZ, an Emergency Control Scheme – Managing *Salmonella* Enteritidis in Commercial Chicken Flocks (Order) was imposed (MPI, 2022). As in Australia, up until recently SE had not been detected in NZ commercial chicken flocks. The emergence of SE in NZ from a poultry source was traced back to late 2019, with no reported SE isolations related to poultry in NZ reported before this incursion (Mulqueen, 2021). As a result of SE detections on several chicken farms in NZ in March 2021, the Emergency Control Scheme (ECS) was developed to manage the risks of SE by strengthening the current controls, verifications and testing levels. From the 6th of October 2021, commercial chicken (*Gallus gallus domesticus*) operators were required to comply with the ECS. Operators include breeders, hatcheries, rearers, broilers, layers and processors. The ECS does not cover eggs for farms with 100 or less laying hens selling eggs direct to consumers. The ECS was active for 6 months. An amended and extended version of the ECS came into force on the 6th of April 2022, which includes a small number of changes based on feedback gathered during implementation of the first iteration of the ECS. The ECS imposes similar requirements as the NSW Biosecurity (*Salmonella* Enteritidis) Control Order on operators to develop, implement and maintain a system that includes documented procedures for biosecurity controls and to start routine sampling and testing. However, the ECS also imposes requirements around vaccination with a vaccine that provides some protection against SE for:

a) parent breeder birds, by point-of-lay; and

b) layer birds, by point-of-lay

The ECS also recommends that great grandparent and grandparent breeders of both layers and broilers and broiler day-old chicks are vaccinated. Currently AviPro® Megan® Vac 1 (ACVM registration number A007935) is the only vaccine approved for SE in NZ under the MPI's *Agricultural Compounds and Veterinary Medicines Act 1997*. AviPro® Megan® Vac 1 is a live vaccine (Elanco, 2021) and according to the product label is recommended for use in broiler, layer and breeder chickens as an immunological aid in the reduction of ST, SE and SH colonisation of the digestive tract and internal organs (MPI, 2022). The vaccine is also stated to be an immunological aid in the reduction of ST and SE colonisation of intestinal, visceral and reproductive tract, including egg colonisation (MPI, 2022).

2.4.3 Vaccination

Salmonella vaccines are an integral part of a *Salmonella* control program when used in conjunction with good sanitation, biosecurity and management practices (for a review see (Aehle & Curtiss, 2017; Martelli et al., 2017)). *Salmonella* vaccines for poultry are commonly used in many countries, with the focus on vaccination against the serovars of major public health relevance; SE and ST. Existing commercially available *Salmonella* vaccines for poultry are intended for use against one or both of these serovars. There is little evidence of cross-protection between *Salmonella* serogroups in chickens, but a partial cross-immunity effect between serogroups B (ST) and D (SE) has been suggested (for a review see (Martelli et al., 2017)). The aim of vaccination in poultry is both the prevention and reduction of intestinal colonisation resulting in reduced faecal shedding and egg shell contamination and also in the diminished colonisation of reproductive tissues produced by the induction of an adaptive immune response (Methner, 2013). Therefore, these criteria are generally included in potency testing of vaccines to examine the level of microbiological contamination of caeca, caecal content, cloacal swabs and different internal organs (Methner, 2013). A substantial proportion of efforts to achieve effective SE control in egg-laying flocks focus on the development of vaccines with greater protective efficacy than is currently available (Gast & Jones, 2017). Multivalent vaccines, able to elicit immunity to diverse epidemiologically relevant strains or serotypes, are of particular interest (Gast & Jones, 2017).

Vaccines contain weakened or inactive parts of a particular organism (antigen) that triggers an immune response within the body. There are two types of adaptive immune responses, called humoral immunity and cell-mediated immunity, that are mediated by different components of the immune system and function to eliminate different types of microbes (for a review see (Abbas, Lichtman, & Pillai, 2015; CDC, 2018c; Clem, 2011)). Humoral immunity is mediated by molecules in the blood and mucosal secretions, called antibodies, which are produced by cells called B lymphocytes and can eliminate **extracellular** microbes. B lymphocytes are the only cells capable of producing antibodies. Cell-mediated immunity, also called cellular immunity, primarily functions against **intracellular** pathogens and is mediated by T lymphocytes. T lymphocytes consist of functionally distinct populations, the best defined of which are helper T lymphocytes and cytotoxic T lymphocytes. Helper T lymphocytes activate macrophages to kill phagocytosed microbes, while cytotoxic T lymphocytes directly destroy infected cells. The body keeps a few T-lymphocytes, called memory cells, that go into action quickly if the body encounters the same microorganism again. Live and inactivated *Salmonella* vaccines are the most widely used type of vaccine in layer hens (Jia, McWhorter, Andrews, Underwood, & Chousalkar, 2020). Live attenuated vaccines contain laboratory-weakened versions of the original pathogenic agent. Live attenuated vaccines produce strong humoral (or antibody-mediated) and cell-mediated responses and typically produce long-term immunity with only one to two doses of vaccine. Inactivated vaccines produce immune responses in different ways than live attenuated vaccines. Inactivated vaccines generally only induce antibody-mediated immunity (not cell-mediated immunity) and often, multiple doses of the vaccine are necessary to build up and/or maintain immunity. It is generally accepted that live *Salmonella* vaccines are more effective against both intestinal and systemic infection than are inactivated vaccine preparations, largely because they stimulate both

the cellular and humoral arms of the immune system (Methner, 2013). To maximise protection, vaccination programs that combine live and killed vaccines are often used (Martelli et al., 2017).

A number of factors can potentially limit the efficacy of vaccination (for a review see (Jia et al., 2020)), including the vaccine delivery method (*e.g*. spray, oral or intramuscular injection) and whether the method of vaccine administration is suitable for the extended life of a layer hen. Due to the long-life span of layer hens (75–100 weeks), there is a need to maintain a strong immune response to *Salmonella*. In a study by van de Reep et al. (2018), reduced immunity to SE was observed in 82 week old hens that had been vaccinated with different SE vaccination schemes during rearing. van de Reep et al. (2018) reported that after SE challenge, 82 week old vaccinated and non-vaccinated hens showed little difference in the infection outcome. In both test groups, the majority of liver, cecum, spleen, and follicular fluid samples were positive for the challenge strain (van de Reep et al., 2018). Increasing vaccine immunogenicity or the administration of additional doses could potentially contribute to providing layer hens with extended immunity against *Salmonella* (Jia et al., 2020). Multi-age farms also represent a particular challenge to the effectiveness of vaccination, as older *Salmonella* infected birds in the shed may serve as a continuous source of bacteria for newly arrived pullets (Sharma et al., 2018). The continuous housing of birds in multi-age farms also inhibits thorough cleaning (Jia et al., 2020). Davies & Breslin (2004) reported that in a highly *Salmonella* contaminated environment (due to poor cleaning and disinfection or insufficient control of rodents etc), flock infection and production of contaminated eggs may still occur, albeit at a lower frequency than would be expected in unvaccinated flocks (R. Davies & Breslin, 2004). This highlights the fact that vaccination alone cannot guarantee freedom of *Salmonella*, good hygienic practices and biosecurity measures are essential.

2.4.3.1 Vaccination in Australia

Currently in Australia there are two registered live ST vaccines that are marketed as an aid in the control of *Salmonella* (P. C. Scott et al., 2020). These are Vaxsafe ST (Bioproperties®) and Poulvac ST (Zoetis®) derived from the same vaccine candidate which was generated by disrupting the metabolic gene *aroA*. These vaccines confer some protection against ST and also some protection against SI, *S*. Heidelberg and SE (for a review see (P. C. Scott et al., 2020)). It was estimated that approximately 75% of all commercial layer flocks were vaccinated against ST in 2017–2019 (McLure et al., 2021). None of the flocks on any of the 16 IPs with poultry onsite, were vaccinated against ST (Catherine Fraser, personal communication). Administration of live vaccines to day-old birds has the potential to circumvent early colonisation. Routine vaccination protocols include multiple administration of a live vaccine in pullets, which may initially be primed by coarse aerosol of day-old chickens at the hatchery and/or followed up by several drinking water applications in the field (P. C. Scott et al., 2020). While there are several variations to the current program being implemented, the most common in the Australian layer industry is several live primes followed by incorporating the suspended freeze-dried vaccine in an inactivated vaccine and administration by intramuscular injection (P. C. Scott et al., 2020).

Australia currently has no registered live SE vaccine, due to its previous SE-free status. However, at the time of the NSW SE outbreak, permits existed in Australia for autogenous *Salmonella* vaccines, which include SE. Guidelines for autogenous vaccine permits are published on the Australian Pesticides and Veterinary Medicines Authority (APVMA) website (APVMA, 2021). Autogenous vaccines are individually tailored to a farm and made from organisms isolated on the farm. Autogenous vaccines are prepared in response to a specific and immediate need, usually when a disease problem arises where registered vaccines are not available, or the registered vaccine(s) are ineffective. An autogenous vaccine can only be used on the flock or sites/facilities under common management from which the microorganism(s) was isolated. One regulatory aspect of autogenous vaccines is that while they must be safe, their efficacy does not have to be proven and their use is at the discretion of the requesting veterinarian. It is the responsibility of the prescribing veterinarian to monitor and evaluate the effectiveness of the vaccine in the target species. Following the NSW SE outbreak, Scott et al. (2020) undertook a study to evaluate the protection conferred by

various vaccination programs including or excluding an autogenous SE 7A bacterin[6] (P. C. Scott et al., 2020). Considering the availability of the existing live ST vaccines in Australia that provide some cross-protection, the level of protection of the live attenuated Vaxsafe ST (Bioproperties®) vaccine alone and in combination the SE autogenous vaccine were evaluated against an SE 7A oral exposure. The four vaccination programs included unvaccinated, only live ST, only killed autogenous SE, and a combined program of live ST and killed autogenous SE. At 16 weeks of age, hens were exposed to SE 7A. Cloacal samples were collected from all hens at 3, 7, 14, 21 and 28 days after exposure (DAE). Hens were humanely euthanised at 32 DAE. *Salmonella* cultures of ovaries and caeca from each bird were undertaken. The results demonstrated that the best protection was achieved by the program that included two vaccinations with ST live vaccine at hatch and at 4 weeks of age, followed by two vaccinations with SE 7A autogenous vaccine at 8 and 12 weeks of age. However, this protection was assessed only 5 weeks after the second vaccination with SE autogenous vaccine. The authors noted that if the protection offered is only short term then it may be necessary to handle the birds for vaccination while in lay, which is both expensive and disruptive. Therefore, Scott et al. (2021) undertook a subsequent study to assess the long-term protection of the vaccination program (combining two vaccinations with a live ST vaccine at hatch and 4 weeks of age, and two vaccinations with an SE 7A autogenous vaccine at 8 and 12 weeks of age), in the reduction of SE 7A faecal shedding and colonisation of caeca, air sacs and ovarian tissue (P. C. Scott et al., 2021). Siblings of the hens included in the first stage of the study (P. C. Scott et al., 2020) were challenged at 47 weeks of age, 35 weeks after the second vaccination with SE autogenous vaccine. In total, the study included 48 laying hens, divided into three groups of 16 birds (unvaccinated/negative control, vaccinated, unvaccinated/positive control). The media used for mock-inoculation of the negative control group was tested to be sterile. Scott et al. (2021) reported that the vaccination program provided long-term memory immunity until at least 47 weeks of age, with the titres obtained with the enzyme linked immunosorbent assay (ELISA) test demonstrating that the vaccinated hens were consistently higher than those of the unvaccinated controls. While there was a decrease in the titres over time, SE antibody levels remained above the ELISA cut-off threshold value in the vaccinated hens. No tissue samples (caecum, air sac, ovarian surface) were positive for SE in the unvaccinated/negative control. Caecal colonisation was 56% (n=9) in the vaccinated and 69% (n=11) in the unvaccinated/positive control. Air sac samples were positive for 13% (n=2) of the vaccinated group and no samples were positive within the unvaccinated/positive control group. No ovarian follicles were infected in the vaccinated group, while 6.3% (n=1) of the unvaccinated/positive control group had a positive ovarian surface sample. There was no statistically significant difference between the vaccinated and unvaccinated/positive control groups in the proportion of tissue samples positive from either the caecum, air sac or surface of the ovary. In addition, there was no difference observed in faecal shedding in the vaccinated group compared to the unvaccinated/positive control group. Scott et al. (2021) proposed that the lack of a statistically significant difference between the vaccinated and unvaccinated/positive control group results, may be due to older hens having a naturally higher level of resistance to *Salmonella*. Scott et al. (2021) concluded that the vaccination program was capable of inducing a humoral immune response that remained at levels above the cut-off 35 weeks following the last booster and that the level of immunity induced by the vaccination program continued to prevent follicular infection, as noted in their earlier study (P. C. Scott et al., 2020). However, as noted by Scott et al. (2021), the low follicular isolation rate from the unvaccinated/positive control group highlights the difficulties in reproducing consistent infection levels in experimental birds. This issue was compounded by the necessity of using small numbers of birds when experimenting with zoonotic organisms in laboratory isolators. Scott et al. (2021) concluded that while the experimental constraints of their study provided more challenging conditions to demonstrate the efficacy of the vaccines, that vaccine protection outcomes were expected to be more favourable under field conditions.

[6] A bacterin is a killed bacterial product administered to immunise the host against a specific bacterial disease.

Clark et al. (2021) investigated the effectiveness of the live attenuated Vaxsafe ST (Bioproperties®) vaccine to provide cross-protection against the Australian SE 7A strain following the administration of the registered vaccination regime and a regime involving an extra injection (Clark et al., 2021). Vaxsafe ST (Bioproperties®) is registered for use by administration at day old by spray application, 14 days via drinking water, 6 weeks of age via drinking water and 12 weeks of age via intramuscular vaccination. The study aimed to determine whether the addition of an intramuscular dose at 14 weeks (i.e. a regime involving a double injection) could provide enhanced cross-protection. Day-old layer chicks were allocated with 20 birds as unchallenged controls and 32 birds in each challenged group receiving either a vaccination regime consisting of a single-injection or a double-injection. The vaccination regimes were applied at one day of age (coarse spray), and 3 weeks age (oral gavage), followed by either one or two intramuscular doses of the same vaccine at 9 or 9 and 14 weeks of age respectively. The birds were challenged orally with the SE 7A at 17 weeks. Clark et al. (2021) reported that all of the challenged groups had a similar proportion of cloacal swabs positive at 3 days post challenge (83%), but that this significantly declined by day 10 and 14 post challenge. Compared with the challenged controls, the proportion of birds with positive cloacal swabs at 5 days post challenge was significantly lower in the single-injection vaccinated group and at 7 days post challenge in the double-injected vaccinated group. There were significant differences in detection from liver or spleen between both vaccinated groups and the unvaccinated group at 7 days post challenge. However, there were no differences between any groups in liver and spleen detection at 14 days post challenge. There were no differences in caecal positive proportions at 7 days post challenge. On day 14 post challenge, the group which had received two injection doses of the vaccine had a lower proportion of positive caeca than the unvaccinated group, whereas the single injected vaccine group did not differ from the unvaccinated group. Reproductive organs showed a low level of colonisation in both the unvaccinated and vaccinated groups, hence significant differences were not discernible. Clark et al. (2021) concluded that partial protection was provided using two vaccine doses administered by injection.

An application to import several live attenuated *Salmonella* vaccines (AviPro® Salmonella Duo; live SE/ST mix, AviPro® Salmonella Vac E; live SE, AviPro® Salmonella Vac T) into Australia is also currently under assessment (DAWE, 2020a). These vaccines are for use in chickens (breeders and layers) and turkeys (breeders and meat production). The indicated use of these *Salmonella* vaccines is for active immunisation of chickens to reduce faecal excretion and colonisation of internal organs with SE and ST and to reduce colonisation of eggs with SE (DAWE, 2020a). All three vaccines are listed in the Code of Practice for Lion eggs (BEIC, 2013a) for authorised use in the UK and are listed to protect against SE and ST (AviPro® Salmonella Duo), SE (AviPro® Salmonella Vac E) and ST (AviPro® Salmonella Vac T) (see Section 2.4.3.2).

2.4.3.2 Vaccination in other countries

As previously mentioned, the nature of public health interventions often means that evaluating their impact is complex as they are often implemented in combination and/or simultaneously. However, the temporal relationship between vaccination programs and the reduction in human disease, for example in the UK, suggests that these programs have made a major contribution to improving public health (Lane et al., 2014; O'Brien, 2013). Lane et al. (2014) reviewed national surveillance data on human salmonellosis in England and Wales during 1945–2011. During this period, >740,000 laboratory reports of *S.* enterica infection were received; almost 330,000 (43%) were for SE. The reporting patterns show that the epidemiology of this pathogen can be divided into 4 stages: pre-epidemic (1945–1981); emergence (1982–1987); epidemic (1988–1998); and decline (1999 onwards). During the emergence stage, the percentage of salmonellosis cases reported in England and Wales due to laboratory-confirmed SE rose from 9% in 1982 (1,099 reports) to 33% in 1987 (6,746 reports). In 1988, SE supplanted ST as the most commonly reported serotype. SE accounted for more than half of all salmonellosis cases for all of the epidemic stage (1988–1998). In 1997, reporting of SE accounted for 70% (23,231 reports) of all salmonellosis cases. During the decline stage, the share of salmonellosis attributable to SE fell from 60% in 1999 (10,827 reports) to 28% in 2011 (2,566 reports). Lane

et al. (2014) report that the decline of the pandemic correlated with the introduction of a vaccination program, enhanced farm hygiene, and management standards implemented through a farm assurance scheme for major egg layer flocks in 1997. Despite its sharp decline during the final years of the surveillance period, reporting of SE has remained above the levels observed during the pre-epidemic stage. Over 90% of UK eggs are currently produced under the British Lion scheme, which mandates that all birds must be vaccinated against SE and ST (British Lion eggs, 2021a). The Code of Practice for Lion eggs (BEIC, 2013a) lists four available live vaccines (AviPro® Salmonella Duo; live SE/ST mix, AviPro® Salmonella Vac E; live SE, AviPro® Salmonella Vac T; live ST, Gallivac SE; live SE) and two available killed vaccines (Gallimune SE+ST; killed SE/ST mix, Nobilis Salenvac T; killed SE/ST mix). As already mentioned, vaccination is not fully protective and there have been a number of recent cases of the Food Standards Agency (FSA) in the UK issuing precautionary advice to consumers who have purchased British Lion eggs which may be contaminated with *Salmonella* (FSA, 2019, 2020). Data from the UK in 2012 showed that the level of *Salmonella* of public health significance in laying flocks, while low, was around 0.07% and has purportedly remained very low (British Lion Eggs, 2021b). However, annual data reported for non-typhoidal *Salmonella* in England, has shown that SE was responsible for the largest number of cases of all serovars from 2017 to 2019 (UK Health Security Agency, 2021a, 2021b, 2021c). Many of the foodborne outbreaks in which SE was the causative agent, were linked to the consumption of eggs. However, serotypes reported by a region are not necessarily circulating locally and may have been imported through travel or traded foods. Around 15% of eggs are imported into the UK (British Lion Eggs, 2021c) and may not have been produced to equivalent standards of welfare and hygiene as those produced in the UK.

In Europe, vaccination against SE for laying-hen flocks is no longer obligatory under European Union (EU) regulations in any member state, as long as a flock prevalence of 10% for *Salmonella* is not exceeded (EFSA Panel on Biological Hazards et al., 2019). In laying hen flocks, prevalence targets are set for the target serovars SE and ST (including its monophasic variant). The prevalence target depends on the prevalence level of the preceding year. In 2016 the prevalence target for all commercial-scale adult laying hen flocks in the production period was set to 2% or less for all member states, except for Poland where it was set to 2.56% or less. In analysing the prevalence data reported by the member states from 2008-2016, EFSA (2019) report that there had been an increase in the flock prevalence of the *Salmonella* target serovars in laying hens since 2014 (exclusion of data from Poland did not change this trend). Reported data for flocks of laying hens in 2016 show that seven member states exceeded the 2016 flock prevalence targets of 1.3% for SE and ST, while during 2015 only one member state (Poland) did not meet the set prevalence target of 0.9%. However, 15 member states exceeded a prevalence level of 1% during 2016, including some of the major egg producing and exporting countries. EFSA (2019) has raised the concern that changes in vaccination programmes used in the EU for laying-hen flocks against SE, now that vaccination for SE is no longer obligatory under EU regulations in any member state as long as the flock prevalence target is not exceeded, may play a role in the increasing occurrence of SE. Similarly, EFSA (2019) propose that complacency in implementation of biosecurity measures such as pest control and terminal hygiene between flock cycles may have followed the initial success of *Salmonella* control programmes which were originally implemented, leading to a greater risk of introduction or recurrence of infection. EFSA (2019) recommended that further investigation of these aspects is carried out, including gathering more detailed information on the diverse vaccination practices in different EU member states. The surveillance data of layer flocks in Europe described above, combined with recently reported foodborne outbreaks in Europe (Dallman et al., 2016; Pijnacker et al., 2019), indicate that SE infection remains a problem in egg production in many EU member states. Indeed, from 2015 - 2018 an outbreak of SE linked to contaminated eggs from Poland, caused the largest known salmonellosis outbreak in Europe, resulting in 1,209 reported cases in 16 countries (Pijnacker et al., 2019).

In the USA, the Food and Drug Administration (FDA) does not mandate *Salmonella* vaccination. The literature review undertaken to inform this risk assessment did not reveal any recent government or industry data on the level of voluntary vaccination within the egg industry in the USA. However, in 2013, the United States Department of

Agriculture (USDA), as part of their National Animal Health Monitoring System (NAHMS), conducted a study of farms in the USA that produce table eggs (for an overview see (USDA, 2014a, 2014b)). A sample of farms with 3,000 or more laying hens was selected from the FDA list of registered egg producers. A total of 328 farms located in 19 States participated in the study. Layers on 89.9% of farms had been vaccinated against *Salmonella* as pullets only, and birds on 9.1% of farms had been vaccinated against *Salmonella* as pullets and again as layers. Only 1.0% of farms had birds that had not been vaccinated as either pullets or layers. Many different vaccination protocols were used. For farms in which birds had been vaccinated against *Salmonella* as pullets, 64.8% had birds that were vaccinated three times as pullets. For farms in which birds had been vaccinated against *Salmonella* as layers, 81.5% vaccinated layers just once. For farms in which birds had been vaccinated against *Salmonella* as pullets, the highest percentage (39.0%) were given two live ST vaccines via spray, followed by an SE bacterin injection. For farms in which pullets had been vaccinated against *Salmonella*, 70.7% had birds that were given an SE bacterin as pullets. SE bacterin was not given to layers. No publicly available information could be found as to whether vaccination practices in the USA have changed since this time. There have been recent outbreaks involving SE-contaminated eggs in the USA (CDC, 2018b). According to data from the Laboratory-based Enteric Disease Surveillance (LEDS) system, which contributes to the understanding of human salmonellosis in the USA by collecting reports of infections from state and regional public health laboratories, there has been a 16.8% increase in the number of reports of SE from 2006 to 2016 (CDC, 2018a). While reporting to LEDS is voluntary and the number of laboratories submitting reports varies somewhat from year to year, almost all laboratories report every year (CDC, 2018a).

2.4.4 Flock testing

SE infects poultry without causing overt disease. During the SE outbreak in NSW, 81% (13/16) of the infected poultry containing premises had flocks with no clinical evidence of disease (Catherine Fraser, personal communication). Of the 3 (19%) infected premises with poultry where clinical signs were detected, symptoms included depression, increased mortalities associated with peritonitis and an increase in vent pecking and cannibalism. These clinical signs were seen in older birds (ranging from 63 -100 weeks), barn style flocks and free-range flocks with a small range area. Mortalities in older birds increased over a 2-3-week period, peaking at around 3 weeks before starting to decline. However, as the properties were destocked, it is unknow what would have happened beyond this time. Clinical signs were not observed in younger birds even when associated with multiple SE positive environmental or serological samples, or when serologically positive in a cage system or free-range system that enabled grazing over a larger range area. Scott et al. (2020) also reported that during the laboratory-controlled exposure of birds to the Australian isolate SE 7A, that no birds demonstrated any overt clinical signs or morphological pathology. Mortality, morbidity and/or production parameters therefore cannot predict if a flock is SE positive. In addition to the lack of clinical signs in infected poultry, compounding matters is the complex network of movement of people, vehicles, and equipment between premises and the time lag between introduction of SE into a premises and its establishment and detection on farm. Surveillance testing for SE is therefore an essential component for effective SE control, both to identify flocks that pose a threat to public health and to verify the cost-effectiveness of resource investments in risk reduction (Gast, 2007).

Testing to decide the fate of flocks or eggs involves considerable uncertainty because of both assay sensitivity limitations and fluctuations over time in the prevalence of SE in the environment, hens, and eggs (for a review see (Gast, 2017; Waltman, 2017)). Effective sampling strategies are therefore critical in enabling the detection of SE infection in flocks. The detection of SE in laying house environments is epidemiologically correlated with the production of contaminated eggs. Because of this relationship and the infrequency at which contaminated eggs are typically produced by infected flocks, testing environmental samples for SE is often the primary screening method used to identify potentially infected flocks for further scrutiny (Gast, 2007; Trampel, Holder, & Gast, 2014). Among environmental testing options, dust samples have been shown to yield both more frequent isolation of SE and a longer

duration of positive results than faecal samples (Arnold, Martelli, McLaren, & Davies, 2014; Gole, Caraguel, Sexton, Fowler, & Chousalkar, 2014). However, even environmental testing on SE positive premises can return a larger percentage of negative results. For example, the percentage of detections on SE premises within NSW from environmental samples was 4.4% (2/45) at IP 1 and 44% (4/9) at IP 4 (Catherine Fraser, personal communication). Bird testing, although not recommended as a screening test, is still used as a confirmatory test. In line with the relatively low SE detection rate observed from environmental sampling in NSW, serological testing of flocks on SE positive premises returned a positive result of 2.2% (2/90) at IP 1 and 19% (34/180) at IP 4 (Catherine Fraser, personal communication). The presence of SE in the edible internal contents of eggs is the most unequivocally relevant measure of the public health risk posed by laying flocks. However, even in flocks known to be infected with SE, the infrequent, sporadic, and transient occurrence of egg contamination limits the diagnostic sensitivity of egg testing (Gantois et al., 2009).

To determine *Salmonella* burden in chicken farms, culture methods of environmental samples that require a turn-around time of 5–7 days are typically used (Ahaduzzaman, Groves, Walkden-Brown, & Gerber, 2021). However, methods of rapid detection of *Salmonella* spp. in environmental samples have been developed (Ahaduzzaman et al., 2021). In addition, a number of studies have focused on the development of serotype-specific assays, to enable the detection of not only *Salmonella* in general, but specific serotypes, without the necessity of isolation. For example, Zhang et al. (2020) developed and evaluated a multiple cross-displacement amplification (MCDA) assay targeting serovar-specific genes for rapid, accurate identification and serotyping of the five most dominant *Salmonella* serovars in Australia: Typhimurium, Enteritidis, Virchow, Saintpaul and Infantis (Zhang, Payne, Wang, Sintchenko, & Lan, 2020). Seven MCDA assays were developed, which enabled detection in as little as 8 minutes and had a limit of detection of 50 fg per reaction (10 copies) from pure DNA. The authors of the study noted that further validation work is required to determine whether the MCDA assays developed can offer a rapid, accurate, and sensitive serotyping method for culture-independent serotyping of common *Salmonella* serovars directly from environmental samples (Zhang et al., 2020). As research into serotype-specific assays for environmental samples matures and moves from the laboratory to the field, the turn-around time for onsite testing will reduce drastically.

In countries in which SE is endemic, substantial resources from both government and private industry have been invested in comprehensive SE testing and risk reduction programs for egg-producing poultry. Sustained participation in these efforts has led to reported declines in both egg contamination and human illnesses in many countries (O'Brien, 2013).

2.4.4.1 Flock testing in NSW

The Biosecurity (*Salmonella* Enteritidis) Control Order 2019 was amended on the 30th of June 2020 to include a requirement for all licensed egg business in NSW to undertake mandatory SE testing from the 1st of July 2020. The Control Order in NSW is in effect until the 30th of June 2024. All SE cases detected in NSW prior to the 1st of July 2020 were from layer flocks that were not conducting routine on-farm testing for SE, underlining the need for these amendments (NSW DPI, 2020b).

Under the Control Order, pullet rearing facilities, breeder farms (excluding those that produce and sell eggs for consumption) and chicken meat farms are not required to conduct sampling and testing for SE. This mandatory SE testing requires sampling and testing of individual sheds/poultry housing areas every 12 to 15 weeks for the duration of the Control Order. The number of samples collected, and the location of sample collection is important. Therefore, farms must implement environmental sampling of poultry sheds/bird housing areas in line with recognised industry procedures. NSW DPI has developed guidelines for sampling. These guidelines reflect industry practice for SE sampling, such as Australian Eggs Standard Operating Procedures (SOPs) for SE sampling. Alternatively, licensed egg business can choose to instead participate in the National *Salmonella* Enteritidis Monitoring & Accreditation Program (NSEMAP), which has more specific sampling requirements.

The NSEMAP provides an accreditation process to demonstrate that all flocks are free from SE. Accredited status is achieved after completion of a two-stage monitoring process which requires three consecutive monthly SE tests (Monitored Status—Stage 1) followed by three consecutive 3-monthly tests (Monitored Status—Stage 2). All SE tests must be negative. For NSEMAP accredited farms sampling must be done in accordance with NSEMAP Guidelines (DAWR, 2021). Various aspects of sample collection for different systems (i.e. cage, barn) are described, including how to collect an environmental swab sample, where to sample and how to pool samples. Positive culture of SE from environmental drag swabs requires confirmatory testing that may consist of serological tests, necropsy and bacteriological culture from animal tissues of the initial sampled flock and further drag swabs (DAWR, 2021). Accredited status also requires that replacement pullets are derived from either an NSEMAP accredited flock, or that the replacement pullets' environment is tested 1 month before arrival according to the NSEMAP Guidelines or, that fifteen blood samples from the replacement flock are tested by the SE ELISA (to achieve a 95% confidence of a 5% incidence) within 1 month prior to arrival with negative results (DAWR, 2021).

Prior to the Biosecurity (*Salmonella* Enteritidis) Control Order 2019 being issued, approximately 75% of NSW layer hens were already being tested for SE under voluntary quality assurance programs like the NSEMAP (NSW DPI, 2020c). Bringing this figure to 100% would be a significant step toward eradicating the threat SE poses to the NSW egg industry (NSW DPI, 2020c). In NSW, SE is notifiable under the *Biosecurity Act 2015* (see Section 1.3.3.1) and following the introduction of the Control Order there has not been any new SE notifications from the environmental testing.

2.4.4.2 Flock testing in other countries

Many countries have implemented microbiological testing requirements. Amongst the most comprehensive and prescriptive, are testing requirements laid out in the Code of Practice for Lion Eggs (2013) in the UK. Testing requirements are set out for *Salmonella* on breeder pullet rearing farms and on transfer (*e.g.* breeder pullet transport vehicle), breeder laying bird farms and hatcheries, pullet rearing farms, laying bird farms and, packing centres. If SE, ST, SI, SH or SV is isolated from the breeder pullet rearing flock or its environment or the breeder laying bird flock or its environment, BEIC must be notified immediately. If an exotic *Salmonella* serovar other than SE, ST, SI, SH, SV is isolated veterinary advice must be sought and advice acted upon. If non-vaccinal SE or ST is isolated from the pullet flock or its environment or the laying flock or its environment, BEIC must be notified immediately. If an exotic *Salmonella* serovar other than SE or ST is isolated, veterinary advice must be sought and advice acted upon. If non-vaccinal SE or ST is isolated from the laying flock, or its environment, BEIC must be notified immediately.

Breeder pullet rearing farms must be sampled after cleaning and prior to a new flock going into the house, from six areas (floors, walls, high beams/ledges and pipe-work, fans and fan housing, hoppers and feeders and drinkers). Additional testing requirements are laid out if the previous flock tested positive for SE, ST, SH, SV or SI. Requirements are also laid out for testing day-old chick delivery box liners (selection of samples to be representative of all source flocks) and all dead on arrival (DOA) chicks and culls at day-old to a maximum of 60 from each hatchery. The flock must then be tested at 4 weeks of age (a minimum of 2 pairs of boot swabs per house or 50g of dust collected from at least ten locations in each house). On transfer (*e.g.* breeder pullet transport vehicle), *Salmonella* swabbing of the transport vehicle(s) must be undertaken to include crates/modules, the vehicle's body floor and driver's foot-well.

Breeder laying bird farms must also be sampled after cleaning and prior to a new flock going into the house, from each of the six areas specified above for breeder pullet rearing farms (*i.e.* floors, walls, high beams/ledges and pipe-work, fans and fan housing, hoppers and feeders and drinkers). Additional sampling must be undertaken to include nest boxes and egg delivery belts. Additional testing requirements are laid out if the previous flock tested positive for SE, ST, SI, SH or SV. Every three weeks, five pairs of boot swabs must be sampled per house (pooled into a minimum of 2 pools per flock). Flock testing must commence at 24 weeks of age and must be undertaken every six weeks (50g of dust collected from at least ten locations in each house). Official sampling must also be undertaken near the beginning

of lay and near the end of lay in alignment with Commission Regulation 200/2010, which states that sampling must be undertaken: '*at the holding on two occasions at any times which are sufficiently distant in time from each other during the production cycle*'. The Code of Practice for Lion Eggs (2013) outlines that official sampling should take place two times during lay; early lay and late lay. Sampling is to consist of five pairs of boot swabs per house (pooled into minimum of 2 pools per flock). Hatcheries are also to be sampled by testing a minimum of ten hatcher tray liners. If SE or ST is isolated from the hatchery (environment), the hatchery must notify the BEIC immediately. If a *Salmonella* serovar, other than SE or ST, is isolated from the hatchery (environment), veterinary advice must be sought and advice acted upon.

Pullet rearing farms must also be sampled after cleaning and prior to a new flock going into the house, from each of the six areas specified above for breeder pullet rearing farms and breeder laying bird farms. Additional sampling must be undertaken on all available rodent faeces (up to 25g from surfaces in house, or from the service area if none is detected in house). If no rodent faeces is detected, the rearer must sign a declaration and take swab or sponge samples from areas around the bait boxes. Additional testing requirements are laid out if the previous flock tested positive for SE or ST. A minimum of ten hatcher tray liners must be sampled from the hatchery and be representative of all source flocks in each delivery. In addition, a maximum of 60 DOAs from each hatchery/delivery must be sampled. Sampling of the litter and cages must take place two to three weeks prior to transfer (approximately 14 weeks). Litter sampling must include a minimum of 2 pairs of boot swabs per house, or composite faecal samples (60 x 1g) (one composite) per house and 50g dust collected from at least ten locations in each house (one composite per house). Sampling of cages includes composite faecal samples compositing naturally mixed faeces from all belts, or scrapers after running, to produce a 60g composite (1 composite) per house and 50g dust collected from at least ten locations in each house (one composite per house). If SE or ST is isolated from a hatcher tray liner or chick box liner, the site must notify their chick supplier and BEIC immediately.

Laying bird farms must be sampled after cleaning and prior to a new flock going into the house, from each of the seven areas specified above for pullet rearing farms. An additional two sites are also specified for sampling, including manure belts/droppings boards and scratching areas and, egg delivery belts/elevators. Additional testing requirements are laid out if the previous flock tested positive for SE or ST. All flocks on site/holding must be tested commencing at 22-26 weeks (154-182 days) of age, every 15 weeks (105 days). The Code outlines specifications for sampling single and multi-age houses. In single age housing, litter is to be sampled by pooling two pairs of boot swabs or socks per house. Cages are to be sampled by taking two 150g faecal samples (pooled for culture) per house from the end of belts, scrapers or pits. In multi-age houses, where an 'all in all out' system is not in operation, the number of boot swabs/socks or faeces samples to be taken is two pairs of boot swabs or socks, or two pooled 150g faecal samples per house from the end of belts, scrapers or pit.

The packing centre environment must be sampled every three months and must include samples from all egg contact surface areas. If *Salmonella* spp. is isolated as a result of post cleaning for *Salmonella*, BEIC must be informed immediately. Egg sampling must be conducted every three months and must include testing of the eggshell and contents. All laying flocks must be monitored for *Salmonella* and must include samples of at least 20 eggs per quarter per farm. In the event of a positive test for SE or ST, BEIC must be informed immediately.

Version 8 of the Lion Code of Practice is currently being prepared, but at the time of writing is not yet publicly available (BEIC, 2021). However, it has been flagged that a number of amendments have been made to the current version of the Lion Code of Practice (Version 7), in terms of requirements around sampling frequency and egg testing (BEIC, 2021).

In the United States, regulatory authority over shell production is defined by a federal rule for prevention of SE in shell eggs during production, storage and transportation (FDA, 2021c). This regulation specifies a list of mandatory risk reduction practices plus a required testing program for egg-laying flocks. Egg producers must first implement a written

SE prevention plan. Egg producers must procure pullets that are SE monitored from certifiably uninfected breeder flocks or raise pullets under SE monitored conditions. “SE monitored” means the pullets are raised under SE control conditions that prevent SE and environmental testing for SE is conducted at 14–16 weeks of age before the birds are transferred into laying houses. On laying farms, environmental samples must be collected and tested for SE at 40–45 weeks of age and again 4–6 weeks after induced moulting. The FDA (2017) set out an environmental testing method to verify that an on-farm egg safety program is functioning in the reduction of SE in laying hen houses. Manure is listed as the preferred sample type and moistened gauze pads are to be used to sample manure the entire length of all rows/banks of the hen house (FDA, 2017). If an SE-positive environmental test is returned at any time during the life of a flock, eggs must be diverted to treatment for the life of the flock in that positive poultry house. Treatments include those that achieve at least a 5-log destruction of SE for shell eggs (*e.g.* pasteurisation). Four egg tests must be conducted on the flock in the positive poultry house at 2-week intervals. A minimum of 1,000 intact eggs must be tested at 2-week intervals for a total of 4,000 eggs. If all four tests are negative for SE, testing of that flock can be reduced to one egg test per month. A minimum of 1,000 intact eggs is to be tested per month for the life of the flock. If the monthly egg tests are negative for SE, supply of table eggs to the market can resume. If an environmental test or an egg test was positive for SE at any point during the life of a flock, prior to depopulation, cleaning and disinfection of the pullet house must include removal of all visible manure, dry cleaning (i.e. remove dust, feathers and old feed) and disinfection with spray, aerosol, fumigation or another appropriate disinfection method.

Like the USA, Canada does not mandate *Salmonella* vaccination. Egg production in Canada is nationally regulated and SE control and prevention programs involve testing throughout the supply chain, from breeder flocks, hatcheries, pullet barns, and layer barns to ensure that SE-positive flocks are quickly identified and the appropriate actions taken (Korver & McMullen, 2017). Environmental swabs are collected from each flock once in the pullet barn (between 3 and 15 weeks of age), early in lay (between 19 and 35 weeks of age) and late in lay (between 36 and 60 weeks of age) by qualified egg board staff. At least 60 different sites within the barn must be collected, with a focus on dust and egg moving equipment and composites. Samples from up to 15 different sites may be pooled for analysis, with a minimum of four pooled samples to be tested. If rodent droppings or dead insects are found, they must also be sampled. Swabs are taken from sites including walls, floors, fans, egg belts, manure belts and coolers. The samples are tested at an accredited laboratory using a culture-based method approved by the Chief Veterinary Officer of each province. If the pullet barn tests positive for SE, the flock is humanely euthanised and the carcasses disposed of in an approved manner (composting, burial or incineration) according to provincial rules and regulations. If the layer barn environment tests positive, all eggs are diverted to a breaking plant, and the product pasteurised until a decision is made regarding the disposition of the flock. Depending on market circumstances, a flock testing positive for SE will be humanely euthanised, and the carcasses disposed of in an approved manner (composting, burial, or incineration) according to provincial rules and regulations. If the flock is allowed to remain in production, all eggs are diverted to a breaking facility and the product pasteurised until the end of life of the affected flock. Once the flock has been depopulated, the barn is cleaned and disinfected according to standard procedures and the barn must be tested again for SE. Individual provincial boards may recommend additional actions.

In Europe, the EU regulation no. 2160/2003 requires member states to take effective measures to detect and control *Salmonella* serovars of public health significance at all relevant stages of the poultry production chain through a national control program. The implementation of this regulation makes strict sampling schemes mandatory in the member states to provide information about *Salmonella* flock contamination. In accordance with regulation no. 2160/2003, prevalence targets have been set for *Salmonella* in flocks of breeding hens, laying hens, broilers, breeding turkeys and fattening turkeys (for a review see (EFSA Panel on Biological Hazards et al., 2019)). The prevalence targets are set on two serotypes (SE and ST, including monophasic ST with the antigenic formula 1,4,[5],12:i:-), except for breeding hens for which the target also includes SH, SV and SI. A number of trade restrictions have been introduced in cases in which these populations are still infected with SE or ST. The regulations lay down testing

schemes (frequency of sampling, sampling protocol, laboratory analysis, reporting requirements) for each of these populations in regard to all identified *Salmonella* serotypes with public health significance. Breeding flocks must be sampled at the initiative of the food business operator and as part of official controls (EU, 2010). A sampling framework is in place to detect the presence of the relevant *Salmonella* serotypes of adult breeding flocks of *Gallus gallus* comprising at least 250 birds. Sampling by the food business operator must take place every two weeks at the place designated by the competent authority, from either the hatchery or at the holding. If sampling as part of official controls takes place at the hatchery, sampling must consist of: (a) routine sampling every 16 weeks at the hatchery; (b) routine sampling at the holding on two occasions during the production cycle, the first one being within four weeks following moving to the laying phase or laying unit and the second one taking place towards the end of the laying phase, not earlier than eight weeks before the end of the production cycle and, (c) confirmatory sampling at the holding, following the detection of the presence of the relevant *Salmonella* serotypes from sampling at the hatchery. At the hatchery, at least one sample must be taken per breeding flock on each sampling occasion. The sample shall consist of at least: (a) one composite sample of visibly soiled hatcher basket liners taken at random from five separate hatcher baskets or locations in the hatcher, to obtain a total sampling surface of at least $1m^2$; (b) one sample taken with one or several moistened fabric swab(s) of at least $900cm^2$ surface area in total, taken immediately after the removal of the chickens from the whole surface area of the bottom of at least a total of five hatcher baskets and, (c) 10g of broken eggshells taken from a total of 25 separate hatcher baskets, namely 250g in the initial sample, in up to five hatchers with hatched eggs from the flock, crushed, mixed and sub-sampled to form a 25g subsample for testing. If sampling as part of official controls takes place at the holding, routine sampling must be carried out on three occasions during the production cycle: (a) within four weeks following moving to the laying phase or laying unit; (b) towards the end of the laying phase, not earlier than eight weeks before the end of the production cycle and, (c) at any time during the production cycle which is sufficiently distant in time from the previous sampling points described. The procedure set out for sampling at the hatchery is the same when conducted by the food business operator and as part of official controls. Sampling at the holding shall primarily consist of faecal samples and shall aim to detect a 1% within flock prevalence, with a 95% confidence limit. To that effect, the samples shall comprise one of the following: (a) pooled faeces made up of separate samples of fresh faeces each weighing not less than 1g taken at random from a number of sites in the poultry house in which the breeding flock is kept or has access (the number of sites from which separate faeces samples are to be taken is specified according to the number of birds kept in the breeding flock); (b) boot swabs and/or dust samples (consisting of five pairs of boot swabs, representing each about 20% of the area of the poultry house and at least one pair of boot swabs representing the whole area of the poultry house and an additional dust sample collected from multiple places throughout the poultry house from surfaces with visible presence of dust) and, (c) in cage breeding flocks, sampling may consist of two samples of at least 150g collected from naturally mixed faeces from either dropping belts, scrapers or deep pits, depending on the type of house. Test results for each member state are reported and must include: the total number of adult breeding flocks comprising at least 250 birds which were tested at least once during the year of reporting; the total number of breeding flocks positive with any *Salmonella* in the member state; the number of breeding flocks positive with at least one of the relevant *Salmonella* serotypes and, the number of positive breeding flocks for each *Salmonella* serotype or for unspecified *Salmonella* (isolates that are not serotyped). For adult breeding hens a maximum of 1% of the poultry population is permitted to be positive for SE, ST, SH, SV and SI. The trade restrictions imposed if this limit is exceeded, include the destruction or safe disposal of (hatching) eggs and birds. Laying flocks are sampled by the food business operator and by the competent authority (EU, 2011). Sampling at the initiative of the food business operator must take place at least every 15 weeks. The first sampling must take place at the flock-age of 24 ± 2 weeks. Sampling by the competent authority takes place in a number of instances, including; (a) at least in one flock per year per holding comprising at least 1, 000 birds; (b) at the age of 24 ± 2 weeks in laying flocks housed in buildings where the relevant *Salmonella* was detected in the preceding flock and, (c) in all other laying flocks on the holding in case SE or ST is detected in one laying flock. The sampling protocol to be used by the food business operator for laying flocks specifies that in cage flocks, 2 x 150g

of naturally pooled faeces that have accumulated on scrapers or belt cleaners must be taken from all belts or scrapers in the house. In the case of cage houses without scrapers or belts, 2 x 150g of mixed fresh faeces must be collected from 60 different places beneath the cages in the dropping pits. In barn or free-range houses, two pairs of boot swabs or socks must be taken for testing. In multi-tier barn or free range houses in which most of the faecal material is removed from the house by dropping belts, one pair of boot swabs must be taken by walking around in littered areas and at least a second pair of moistened fabric swabs must be taken from all accessible dropping belts. The competent authority is to use a sampling protocol including at least one sample collected using the sampling protocol used by the food business operator. Further samples may be taken in order to ensure representative sampling if required by the distribution or the size of the flock. The competent authority may decide to allow replacement of one faecal sample or one pair of boot swabs by a dust sample of 100g collected from multiple places throughout the house from surfaces with a visible presence of dust. As an alternative one or several moistened fabric swab(s) of at least 900cm^2 surface area in total may be used instead to gather dust from multiple surfaces throughout the house, ensuring that each swab is well coated with dust on both sides. Test results for each member state are reported and must include the total number of adult laying flocks which were tested at least once during the year of reporting; the total number of laying flocks positive with any *Salmonella* serotype; the number of laying flocks positive at least once with SE and ST; the number of positive laying flocks for each *Salmonella* serotype or for unspecified *Salmonella* (isolates that are not serotyped) and, explanations of the results, in particular concerning exceptional cases or any substantial changes in number of flocks tested and/or found positive. For adult laying hens a maximum of 2% of the poultry population is permitted to be positive for SE and ST. The trade restrictions imposed if this limit is exceeded, include destruction or safe disposal of hens, marketing of eggs as class B (only for heat treated egg products). At the retail level, European legislation (EU regulation no. 2073/2005) dictates that no *Salmonella* is to be found in 5 × 25g of eggs placed on the market during their shelf-life, otherwise these products are to be eliminated (EU, 2005). Although this strategy would aid in monitoring for highly positive egg batches, the possibility of introduction of *Salmonella* contaminated eggs into the food chain cannot be excluded.

2.4.5 Bird health and spent hens

Standard 4.2.5 addresses diseases or conditions of the layer flock that could make eggs unsafe or unsuitable. However, the health and disease status of birds in breeder flocks, hatcheries and pullet rearing, and spent hens, is not explicitly considered by the standard (FSANZ, 2021a). The definition of poultry in the NSW 2020 SE control order, includes spent hens and fertilised eggs (NSW DPI, 2020a). In relation to spent hens, businesses or persons (including members of the public) receiving ≥100 spent hens must have an appropriate license and a PIC.

In regard to bird health and *Salmonella*, breeder hens have played an established role in the spread of SE globally (Li et al., 2021). Colonised breeder hens can pass on specific serotypes (*e.g.* SE and ST) directly to their progeny via in ovo transmission (Braden, 2006; Liljebjelke et al., 2005). In addition to vertical transmission, horizontal transmission can result in *Salmonella* contamination of poultry hatching eggs *i.e.*, through the environment, via transportation equipment, or by vectors, such as rodents. The hatchery is therefore a central point in the poultry production chain where newly hatched birds can be exposed. Similarly, during pullet rearing, newly hatched chicks are highly susceptible to the establishment of persistent *Salmonella* carriage in the intestinal tract and can shed the pathogen in their faeces for many months and carry it with them to commercial farms (Van Immerseel et al., 2004). The health of breeders, as well as chicks and pullets destined to be egg layers, is important to reduce *Salmonella* colonisation from top-down in progeny and to reduce *Salmonella* prevalence and transmission through the food chain to humans.

Spent hens are commercial layer chickens that are past their optimal egg laying age and are no longer commercially viable. In the Australian egg industry, this typically refers to commercial layers older than 76 weeks in age (Graham, Li, & Hartmann, 2021). Spent layers have historically been caught, crated and transported to various poultry processing plants where they are processed for use in a variety of human food products (Australian Eggs Limited, 2021b). It has

been reported that in recent years, lower prices for the meat of spent hens and its limited uses (mainly used for fertiliser and composting) have resulted in a situation where farmers now pay approximately 25 cents per culled hen (Graham et al., 2021). The cost of culling birds has become an issue for some farmers, with the lower price provided from hen disposal creating an incentive to dispose of birds through alternative means. Industry Guidelines have been developed to assist the Australian egg industry to undertake efficient and welfare-sensitive mass euthanasia of spent layer hens (Australian Eggs Limited, 2021b). However, rather than paying costs for mass destruction, some producers sell their spent hens to backyard or micro-commercial producers to recuperate costs (Graham et al., 2021). Spent hens will continue to lay eggs, albeit with lower productivity and life expectancy. This secondary market in spent hens is largely unregulated, as backyard production is mainly for personal consumption and eggs are not sold. This secondary market in spent hens has the potential to introduce biosecurity risks that could impact the surrounding network of egg producers, as well as consumer safety. At least one study has examined the influence of bird age on the response to SE infection (T. J. Humphrey, Chart, Baskerville, & Rowe, 1991). Humphrey et al. (1991) reported that the age of the bird at infection was found to have an effect on both pathogenesis and antibody response. After direct administration of SE into the crop, birds at 20 weeks of age showed no adverse signs and developed high titres of antibodies. Whereas birds which were 52-55 weeks old at infection developed relatively little antibody and had acute septicaemia, with 6 of 10 birds either dying or having to be humanely destroyed. It was proposed that changes to the immune status of the older birds which could have occurred as a consequence of fatigue associated with intensive laying (T. J. Humphrey, Chart, et al., 1991). Compounding issues is the fact that a large proportion of the producers in the backyard and micro-commercial segment are thought to be hobbyists and therefore, would not be educated on the requirements around flock immunisation or the initial indicators of an outbreak (Graham et al., 2021). However, there is limited data available to estimate the size and extent of this secondary market in spent hens and the impact it may potentially create within these backyard and micro-commercial sectors (Graham et al., 2021). In addition, there is limited research and quantification on backyard and small-scale egg production in Australia (Graham et al., 2021). However, it is generally recognised that backyard and micro-commercial production, which are not captured in most conventional datasets for the egg industry, have an impact on the total number of eggs consumed in Australia (Graham et al., 2021).

Graham et al. (2021) undertook a desktop review of existing assessments of the backyard and micro-commercial producer sectors and consulted with a range of industry stakeholders to collate best estimates of the potential size of each within Australia. There is no formal definition of backyard egg production. Graham et al. (2021) defined backyard producers as households raising less than 15 hens and whose level of production is typically driven to meet personal consumption demands, with additional eggs distributed to friends and family. In comparison, micro-commercial producers are defined as small-scale operations with up to 1,000 hens, whose level of production is substantially greater than observed in the backyard sector, but not to the same scale as larger commercial producers (Graham et al., 2021). As a pullet, the average hen will lay around 200 eggs a year (4 eggs per week) and at around one and a half years of age their eggs become fewer (Australian Eggs Limited, 2022; NSW Agriculture, 2003). Micro-commercial producers that produce no more than 20 dozen (240) eggs for sale in any week, do not need to apply for a NSW Food Authority licence (see Section 1.1). However, they do need to 'notify' the Food Authority with their business details and food activities. Graham et al. (2021) developed a model to estimate the size of the backyard and micro-commercial market within Australia. Their model derived a weighted average estimate of 2.2 million hens in the backyard and micro-commercial market, which account for 28 million dozen eggs. Across the entire range of model estimates (between 174 - 591 million eggs), the backyard and micro-commercial market could account for between 3.0% - 8.6% of Australia’s total egg production (Graham et al., 2021). Of the 2.2 million hens, Graham et al. (2021) estimated that approximately 900,000 are in the backyard sector and approximately 130,000 are in the micro-commercial sector. The authors noted the uncertainty relating to the origin of these hens and whether they are from major hatcheries or from presently uncounted sources such as spent hens or backyard breeding. However, Graham et al. (2021) reported that

their consultations suggested that hundreds of thousands of spent hens are entering the backyard market through this channel.

An increasing trend of households becoming more self-sufficient, accelerated by the COVID-19 pandemic, is one of the potential factors that could factor into significant growth in the non-commercial market (Brown, 2020; Gaffney & Kinninment, 2020). Graham et al. (2021) reported data derived from the Australian Eggs Sustainability Framework survey between 2018 and 2021, which asked respondents whether they keep hens in their household, and if so, how many hens they keep. In the majority of Australian States, apart from South Australia (SA) and WA, the reported number of people keeping hens significantly increased during 2021. In NSW the percentage of people who reported keeping hens increased to 15.6% in 2021, from 8.0% in 2020. The average number of hens kept by those in NSW who reported keeping hens, dropped to 3.7 in 2021, from 8.6 in 2020. When taken together, the data suggests there was a significant increase in the number of households in NSW keeping a small number of hens in 2021, which in turn resulted in lower average figures in the number of hens kept (Graham et al., 2021).

Backyard poultry producers have been associated with outbreaks of endemic (*e.g. Salmonella*) and exotic (*e.g.* Avian Influenza) disease all over the world (Correia-Gomes & Sparks, 2020). In Australia, there have been several recent salmonellosis outbreaks involving backyard chickens. In NSW in September 2019, an ST outbreak occurred involving cake consumed by approximately 35 people at a primary school, with frosting made with raw eggs sourced from backyard chickens (Communicable Diseases Branch, *In print-a*). The class teacher reported that approximately 15 students were ill and away from school the following week. The class teacher and five students were tested and were found to be positive for *Salmonella*. In QLD, ST in backyard chickens was the cause of an outbreak affecting 17 people, including 13 children under 11 years, five of whom were hospitalised (Food Safety Information Council, 2020). In VIC, there were nine cases of SE, five of which were linked to newly purchased chicks (Food Safety Information Council, 2020). There have been very few studies conducted on the management and biosecurity practices of backyard and hobby farm poultry keepers in Australia, or the disease risk they could pose to the commercial poultry sector. Citing an Australian study by Hernández-Jover et al. (2009), Manning et al. (2015) state that the biosecurity practices of small poultry keepers are poor compared to commercial enterprises, in particular hens have high levels of outdoor access, regular contact with wild birds and frequent movement of poultry between backyard sites. Manning et al. (2015) also state that backyard poultry owners have limited contact with veterinarians, which could result in a failure to detect a potential disease outbreak in the early stages (Manning, Gole, & Chousalkar, 2015). Hernández-Jover et al. (2009), investigated the biosecurity practices of producers selling birds through live bird sales in Australia (Hernandez-Jover, Schembri, & Toribio, 2009). The study included those producers selling to bird sales located within NSW, VIC and SA. The producers were selling a variety of different bird species, including egg and meat producing chickens, ducks, turkeys, geese, pigeons, pet birds and show birds. All interviewed producers trading birds were hobby farmers and did not have any contact with commercial poultry farms or poultry processing plants. Almost one third of the producers interviewed (31%; 12/39) had commercial poultry operations within a 5 km radius of their property. Among producers interviewed, the authors noted that there was a poor understanding of biosecurity. Most producers (80%; 31/39), when asked about biosecurity, did not know the meaning of the term, and the general description of biosecurity among producers who had heard of it related to practices on farm to prevent disease entry and transmission. This was reflected in the limited application of on-farm biosecurity practices with no precautions related to footwear (70% of NSW producers), hand washing after contact with birds (20% of NSW producers) or showering before and after contact with birds (90% of NSW producers). Further, some producers did not use an appropriate disposal process for dead birds. Visitors were allowed on farm by all producers (70% of NSW producers), but most producers did not allow direct contact with their birds. In addition, all interviewed producers kept different bird species and a high proportion also kept other animal species. In NSW, 70% of producers also reported keeping ruminants, 10% also kept pigs and 70% had dogs/cats on farm. In another study in Australia, Manning et al. (2015) undertook a study to determine the prevalence of *Salmonella* in 30 backyard flocks in SA (Manning et al., 2015). Out

of 30 flocks, four flocks tested positive for *Salmonella* spp. The overall *Salmonella* isolation rate was 10.4% (12 isolates from 115 samples). The serovars isolated were *S*. Agona, *S*. Wandsbek and *S*. Bovismorbificans. *S*. Agona and *S*. Bovismorbificans are potentially pathogenic and have caused zoonotic food related outbreaks of *Salmonella.* The backyard chickens in all flocks tested were of mixed ages and all birds had contact with other animals such as cattle, sheep, dogs or cats. The authors noted that there is a possibility that chickens may have acquired infection from other farm animals or vice versa. Birds were allowed to range during the day and were locked in a chicken coop/house at night. Many of the backyard flocks from the study were in close proximity to free range commercial broiler and layer farms. The authors stated that the implications of this in regard to horizontal transmission of *Salmonella* warrants further investigation. Keerthirathne et al. (2021) aimed to investigate the presence of *Salmonella*, *Campylobacter* and *Shigella* in Australian backyard poultry flocks and to determine risk factors for these pathogens (Keerthirathne, Ross, Fallowfield, & Whiley, 2021). Poultry faeces samples were collected from 82 backyards and screened for *Salmonella* spp., *Campylobacter* spp. and *Shigella* spp. using qPCR. The 82 samples consisted of 76 samples from SA, four samples from VIC and one sample each from NSW and TAS. Where the authors provided a breakdown of the size of the flocks of the 82 participants, 15 (18%) had flocks of two to three birds, 32 (39%) had flocks of three to five birds and 18 (22%) had flocks which contained more than ten birds. Most of the participants (63%) had multi-aged birds in a flock, and all backyard poultry flocks with more than 10 birds contained multi-aged birds. Of the backyard samples tested, 4% (*n*=3) were positive for *Salmonella* spp. and 10% (*n*=8) were positive for *Campylobacter* spp. and none were positive for *Shigella* spp. None of the samples were positive for more than one pathogen. A higher infection rate was seen in multi-aged flocks (24%) compared with the single-aged flocks (3%). Of the flocks that contained more than 10 birds, 38% were positive for either *Salmonella* spp. or *Campylobacter* spp. This was proposed to be due to all larger backyard flocks (>10 birds) containing multi-aged birds, including older birds. The backyard poultry owners were given a questionnaire to assess their knowledge regarding management of poultry and eggs and to identify potential risk factors that may contribute to the presence of zoonotic pathogens in the flocks. Most of the participants (96%) had no control measures in place for *Salmonella* spp. Only 18% of the participants had their birds confined to one specific area in the backyard, while the remaining 82% allowed their poultry to roam around in the entire backyard. A significant number of participants spread the faeces from their flocks on site (26%), while 49% used the faeces as fertiliser. The survey found that many participants were also engaging in risky food safety behaviours with 46% of participants responding that they washed their eggs with running water or still water instead of wiping the dirt off with a damp cloth, 15% used the eggs as they were (i.e. without wiping the dirt off to clean the eggs), 19% stored their eggs at room temperature and 10% used cracked eggs. Apart from these behavioural patterns, within the study population 26% consumed raw or under cooked eggs. The results of the study demonstrate that backyard poultry may pose a potential risk for salmonellosis and campylobacteriosis. The authors proposed that educating backyard chicken owners on appropriate handling techniques and personal hygiene could help reduce the incidence of foodborne illnesses related to backyard chickens. Additionally, the authors proposed that initiatives in informing the public on having a limited number of birds and to have the birds confined in a specific, spacious area could also help reduce the incidence of foodborne illnesses. These proposals correlate with findings from similar work conducted overseas (Brochu et al., 2021; Correia-Gomes & Sparks, 2020; Kauber, Fowler, Lipton, Meschke, & Rabinowitz, 2017; Nicholson, Campagnolo, Boktor, & Butler, 2020; Pires et al., 2019; Souvestre et al., 2021), which also recommend education and outreach targeting backyard flock owners to improve husbandry and hygiene practices and reduce the risk of zoonotic diseases associated with raising poultry in the backyard setting. Engagement with backyard poultry keepers would not only provide a way to disseminate reliable information generally, but also information about disease outbreaks specifically. Accurate records of backyard flock information (e.g. location, number of birds kept, bird type kept, biosecurity measures implemented, etc.) would also aid contingency planning in an outbreak. Backyard bird owners also play a vital role in preventing disease outbreaks of Avian Influenza (AI) (DAWE, 2019). While there is no evidence to suggest that the consumption of poultry or eggs could transmit the AI virus to humans (OIE, 2021), AI could potentially mutate into a highly pathogenic form capable of transmission by a foodborne route. Ongoing

circulation of various strains (e.g. H5N1, H5N2, H5N8, H7N8) and outbreaks of AI continue to be a global public health concern (OIE, 2021). Furthermore, 2020-2021 saw the most widespread outbreaks of high-pathogenic Avian Influenza (HPAI) in the EU on record (Katselos & Novitski, 2021). The HPAI outbreaks in VIC, Australia in July and August 2020 had a significant impact on the domestic poultry market and Australian avian commodity export markets (Katselos & Novitski, 2021). Australia regained its previous animal health status for freedom from HPAI in accordance with OIE (World Animal Health Organisation) Code guidelines on the 26th of February 2021 (Katselos & Novitski, 2021).

2.4.6 Temperature control for intact shell eggs in the supply chain

The influence of storage temperature on *Salmonella* contamination in and on eggs is a key consideration supporting decisions about egg storage and shelf life. Growth of most salmonellae is substantially reduced at <15°C and prevented at <7°C (FSANZ, 2009b). Refrigeration has been widely recommended as one of the pivotal strategies for minimising the risk to consumers from SE in eggs (Gast, Guraya, Guard-Bouldin, & Holt, 2007). Egg contamination seldom occurs at high frequencies, or involves large numbers of bacterial cells, even when hens are infected with massive oral doses of SE (Gast & Holt, 2000; Gast et al., 2019). Gast et al. (2000) reported that experimentally infected hens laid eggs in which the incidence of SE contamination was 2.5% of yolk and 0.5% of albumen samples. Most of the contaminated eggs contained fewer than 1 SE cell/ml of egg yolk or albumen, and no sample contained more than 67 SE cells/ml (Gast & Holt, 2000). While Gast et al. (2019) reported that the overall incidence of contamination of eggs by SE in experimentally infected laying hens across four commercial genetic lines (consisting of two white egg lines and two brown egg lines) was low, however the incidence in white eggs (3.38%) was significantly higher than among brown eggs (1.56%). The effectiveness of egg refrigeration for preventing the growth of small populations of SE depends on the initial level and location of contamination, the potential for movement of bacteria or nutrients within eggs during storage, and the rate at which growth-restricting temperatures are reached. As previously discussed in Section 2.1.1.1.1.2, 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 (Gast, Guraya, Guard-Bouldin, & Holt, 2008). *Salmonella* deposited in the albumen or on the outside of the vitelline (yolk) membrane, need to be able to survive and grow in this antibacterial environment, before they are capable of migrating to and penetrating the vitelline membrane to reach the nutrient-rich yolk. As previously discussed (Section 2.1.1.1.1.4), studies have shown that the egg can resist SE growth for approximately 2 to 3 weeks at room temperature (T. J. Humphrey, Whitehead, 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, Sakakibara, Konuma, Sawada, & Kumagai, 2001; T. Humphrey & Whitehead, 1993; Messens, Duboccage, Grijspeerdt, Heyndrickx, & Herman, 2004). After the loss of the yolk membrane integrity, the extent of survival of *Salmonella* in albumen and growth upon 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 (Chen, Thesmar, & Kerr, 2005). Storage at 4°C was reported to preserve the antimicrobial agents of the albumen and maintained the integrity of vitelline membranes. Growth of a cocktail 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 cells per egg (Chen et al., 2005). In comparison, egg storage at 22°C led to significant deterioration of the vitelline membranes and SE was reported to flourish, even in the albumen with the lowest initial population (10^2 cells per egg). Similarly, Clay et al. (1991) reported that growth of SE 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 (Clay & Board, 1991). Therefore, for its potential to be effectively realized, chill storage would have to be imposed from shortly after lay until immediately before the cooking and consumption of an egg.

Whiting et al. (2000) developed a model to estimate growth of SE during egg collection, processing, storage and transportation (Whiting et al., 2000). The input parameter values (*e.g*. 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. Whiting et al. (2000) modelled the Yolk Mean Time (YMT), which was (arbitrarily) defined as the storage time when 20% of eggs supported growth in the albumen. Whiting et al. (2000) used growth based on high concentrations of SE inoculated directly into the egg albumen to calculate YMT, as well as SE growth rates in the yolk. Conditions to support growth at a given temperature in an individual egg may vary substantially; thus, large confidence intervals are associated with the YMT predictions. The time for any individual egg to support growth at a given temperature could vary substantially based on, for example, the condition of the egg. The results of the study demonstrated the relative importance of ambient air temperature and indicated the greatest safety improvements in this phase for shell eggs would result from preventing unrefrigerated storage or hastening cooling immediately after lay. Risk assessments for SE in eggs using the Yolk Mean Time (YMT) approach discussed in Whiting et al. (2000), have been conducted in a number of countries including Australia (see Section 2.4.6.1), New Zealand (see Section 2.4.6.2) and the USA (see Section 2.4.6.1).

While refrigeration can help minimise the risk of *Salmonella*, in some countries refrigeration of eggs before sale to the final consumer is not permitted owing to concerns that refrigeration and then gradual warming (*e.g.* from supermarket to fridge at home) creates condensation (see Section 2.4.6.2). Condensation on eggs due to removing eggs from storage at 4°C to ambient temperature, while not a hazard in its own right, provides opportunity for bacterial survival on the shell surface and ingression of potentially contaminated surface moisture into the eggshell. 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, industry quality assurance practices aim to prevent temperature changes that may cause condensation to form on the egg surface. In addition, washing of eggs, if carried out under the appropriate conditions, results in a reduction in the microbial load on the egg surface which minimises the chance of ingression of bacteria into the egg and cross contamination occurring in the kitchen environment. However, wash cycles need close monitoring to assess the effectiveness of reducing *Salmonella* loads on eggs during processing. Washing eggs increases the risk of contamination if the washing is not carried out correctly. For example, if the temperature of the wash water is lower than that of the egg a pressure differential can be created allowing microorganisms that may be present on the shell surface to be drawn into the egg contents. Washing can also damage or remove the protective cuticle of the egg, which increases the risk of bacterial transfer into its internal contents (Australian Eggs Limited, 2021c). In addition, regular cleaning of the egg washing and grading equipment is also necessary to avoid recontamination of eggs after washing.

2.4.6.1 NSW

FSANZ (2011) excluded refrigeration during retail storage as an option during development of Standard 4.2.5, as at the time, SE was not present in Australian laying flocks (FSANZ, 2011). As intact eggs were unlikely to be infected internally with *Salmonella*, there was seen to be no need to refrigerate them to prevent bacterial growth. Australian Egg Industry guidelines recommend that eggs are stored at temperatures of below 15°C as soon as possible after collection and washed within four days of being laid (Australian Eggs Limited, 2021c). However, as there are no prescriptive guidelines on egg storage on supermarket shelves, it is not uncommon to find eggs kept on the shelf at ambient temperature.

Thomas et al. (2006) used Australian production and processing data to examine the impact of different practices on the growth of *Salmonella* in whole shell eggs and to calculate the YMT (Thomas et al., 2006; Whiting et al., 2000). The YMT model is based on data derived from experiments using SE (Whiting et al., 2000) and at the time of their study (Thomas et al., 2006), SE was not considered a hazard for eggs in Australia. Citing earlier literature, which measured the time until ST growth when injected into eggs, Thomas et al. (2006) suggested that YMT does not necessarily

reflect the behaviour of non-Enteritidis serotypes (Cogan et al., 2004). Accordingly, Thomas et al. (2006) used calculations based on predicted ST growth rates to assess egg storage times relevant to Australian egg production conditions and serotypes. To investigate the effect of both on-farm and processing factors, the performance of the best 10%, median 10% and the worst 10% of farms or processors were analysed to develop statistical distributions as model inputs. The potential for growth of *Salmonella* spp. during retail storage is dependent on the remaining YMT at the end of wholesale storage, and times and temperature of storage at retail. Thomas et al. (2006) predicted that storage at 16°C in the retail environment will allow growth of *Salmonella* in contaminated eggs in as little as 18 days after the end of on-farm storage under median industry practices. 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 that eggs commercially produced and graded in Australia may pose a potential risk to consumers if they are stored at 20°C up until the maximum recommended shelf-life before consumption.

Australian Egg Industry guidelines recommend eggs are used before the 'best before' date, which equates to a period of 42 days or less from the time of packing but applies only to eggs maintained at optimal temperature conditions (Australian Eggs Limited, 2021e). However, there are no legal requirements for eggs to have a 'best before' date of 42 days, unless requested by a customer. The 42-day recommendation stems from industry experience and relates to maintaining optimal egg quality, not to egg food safety issues (Australian Eggs Limited, 2021e). The 42-day recommended shelf-life is at least 10 days above that recommended or regulated in the USA, Europe and the UK (see Section 2.4.6.2). In 2015, a nationwide egg producers' workshop was conducted on ST in the Australian egg industry (Chousalkar et al., 2017). The workshop involved more than 80 commercial egg producers and discussion included the need to review the guidelines regarding the Australian table egg best before date, in light of the possibility of ST's survival on the eggshell surface of eggs for up to three weeks (Chousalkar et al., 2017; Gole, Chousalkar, et al., 2014). Gole et al. (2014) reported that five ST strains were capable of survival for up to 21 days on the surface of washed and unwashed eggs incubated at 20°C. These findings were in agreement with findings by De Reu et al. (2006) who reported survival of SE on eggshell surfaces after 21 days of incubation at 20°C (De Reu et al., 2006). Egg washing with sanitisers is one of the most common methods of reducing eggshell contamination. Although egg washing is not a requirement in Australia, sale of dirty eggs is prohibited by FSANZ 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, however variability in egg washing practices across the Australian egg industry were highlighted in the survey results from the nationwide egg producers' workshop (Chousalkar et al., 2017). Most commercial egg washing machines have an egg contact wash time of around 30 s or less (Chousalkar et al., 2017). During the egg washing process, a number of factors are critical in ensuring the effective reduction of *Salmonella* loads on eggs. Amongst these factors are the wash water temperature, egg temperature (external and internal), efficacy of cleaning agents, sanitisers, functionality of egg washing equipment (egg rollers and brushes, spray nozzles), water quality, consistent supply of fresh chemical in egg washing machines, reuse of washing solutions, functionality of dosing pumps, and overall cleanliness of the egg washer (Australian Eggs Limited, 2021c; Chousalkar et al., 2017).

2.4.6.2 Other countries

In NZ, two recent reports have been undertaken by the MPI to investigate the impact of storage times and temperatures on egg-associated *Salmonella* isolates (MPI, 2015, 2019). At the time of publication of these reports, there was no evidence of internal contamination of NZ eggs with *Salmonella*. However, it was considered that there remained a possibility that penetration (horizontal transfer) through the shell resulting from a combination of external contamination and storage and handling conditions could occur. A Risk Assessment was undertaken in 2015 (MPI, 2015) which modelled storage times and temperatures based on YMT calculations using an equation from Whiting et al. (2000). It was assumed that contamination involved a single cell, that contamination occurred post-laying, and used growth rate predictions based on Thomas et al. (2006). Based on the calculations, the total number of days until 20%

of contaminated eggs permitted growth of *Salmonella* was predicted to be 45.9, 28.1, 17.2, 10.5 and 6.5 for storage temperatures of 10, 15, 20, 25 and 30°C, respectively. After this time, logarithmic growth would result in any *Salmonella* present in the egg rapidly reaching levels likely to deliver an infectious dose in a temperature-dependent manner. Based on these calculations, the report recommended that retaining the recommendations for NZ storage times and temperatures would be prudent. The MPI egg risk management programme included the following storage and shelf life options for eggs:

• 21 days where the storage/holding temperature may exceed 15˚C;

• 35 days if stored or held at 15˚C or less.

In a subsequent project, MPI (2019) undertook a study to investigate *Salmonella* survival on the shell and internalisation in chicken eggs in the context of the NZ processing and retail environment. The study focused on addressing whether storage of shell eggs at 15°C or less for a shelf life of 35 days, was necessary to protect consumers of NZ eggs from salmonellosis. The project included a review of new data published since the 2015 Risk Assessment (MPI, 2015) and experiments designed to fill the identified gaps in knowledge by focusing on NZ-relevant *Salmonella* serotypes on eggs at NZ-relevant storage temperatures (focusing on 15°C and 22°C). The conclusion of the report was that for clean, uncracked eggs, 15°C storage does not provide better protection for consumers compared with storage at room temperature (MPI, 2019). Following the detection of SE on poultry farms in NZ in late 2019, MPI advises consumers to keep eggs in the fridge after purchase (MPI, 2021).

In the USA, the FDA does not mandate *Salmonella* vaccination. In order to help prevent the growth of SE in eggs, the FDA ruled that retail establishments must store and display shell eggs under refrigeration at an ambient temperature not greater than 7.2°C (45°F) (FDA, 2021a). As the potential for growth of *Salmonella* in contaminated eggs is dependent on the temperature of the egg from point of lay through to consumption, shell eggs must be held and transported at or below 7.2°C (45°F) ambient temperature beginning 36 hours after time of lay (FDA, 2021c). If the eggs are to be processed as table eggs and are not processed for the ultimate consumer within 36 hours from the time of lay and, therefore, are held and transported as required at or below 7.2°C (45°F) ambient temperature, then they may be held at room temperature for no more than 36 hours just prior to processing to allow an equilibration step to temper the eggs (FDA, 2021c).

In regard to the maximum hold time (36 hours) before a shell egg must be stored at or below 7.2°C (45°F), the FDA was petitioned to change the time to 72 hours after production, in order to better accommodate shell egg production over weekends (FDA, 2009). The 36-hour limit is supported by a model, contained in an SE risk assessment conducted by the FDA and the Food Safety and Inspection Service (FSIS) in 1998 (for a review see (FDA, 2009)), which was developed to examine the relationship between holding time, holding temperature, and yolk membrane breakdown as an indicator of SE risk. The yolk membrane separates the nutrient-rich yolk and any SE that might be present in the albumen. Breakdown or loss of the yolk membrane results in rapid growth of SE present in the albumen. The model showed that, at temperatures that might be observed in unrefrigerated egg holding areas in farms or warehouses or in transport vehicles (70 to 90°F; 21 to 32°C), there was much less breakdown of yolk membrane in eggs held no longer than 36 hours than in eggs held no longer than 72 hours. According to the model, eggs held at 70°F (21°C) would experience at least a 16% breakdown of yolk membrane after 36 hours and a 25% breakdown after 72 hours. While eggs held at 80°F (27°C) would suffer at least a 22% breakdown after 36 hours and a 39% breakdown in the yolk membrane at 72 hours. At 90°F (32°C), there is at least a 33% breakdown after 36 hours and at least a 62% breakdown of the yolk membrane after 72 hours. FDA and FSIS updated this risk assessment in 2005 (for a review see (FDA, 2009)) and refrigeration was modelled again. The risk assessment found that limiting eggs to just 12 hours without refrigeration, the shortest timeframe between laying and refrigeration that was evaluated, provided the greatest public health benefit among the time frames studied.

In the USA, use of either a "Sell-By" or "Expiration" (EXP) date is not a Federal regulation, but may be required, as defined by the egg laws in the state where the eggs are marketed (for a review see (USDA, 2019)). Some state egg laws do not allow the use of a "sell-by" date. Many eggs reach stores only a few days after the hen lays them. About 60% of the eggs sold in the USA come from processors who participate in USDA's voluntary grading service, which ensures that eggs meet the USA grade standards for quality and sanitary processing (USDA, 2021). Government regulations require that USDA-graded eggs be carefully washed and sanitised using only compounds meeting FDA regulations for processing foods. Egg cartons with the USDA grade shield on them must display the "pack date" (the day that the eggs were washed, graded, and placed in the carton). When a "sell-by" date appears on a carton bearing the USDA grade shield, the code date may not exceed 30 days from the date of pack. The FDA (2021) advises consumers to refrigerate eggs at a temperature of 40°F (4.4°C) or below and to use them within 3 weeks for best quality (FDA, 2021d).

As in the USA, in Europe vaccination against SE for laying-hen flocks is not obligatory under EU regulations (as long as a flock prevalence of 10% for *Salmonella* is not exceeded) (EFSA Panel on Biological Hazards et al., 2019). In contrast to the USA, in Europe, eggs are not required to be refrigerated at retail and are not individually marked with use-by dates in most countries (EFSA Panel on Biological Hazards et al., 2019). European legislation in regard to marketing standards for eggs specifically states that: "*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. Therefore, eggs should be stored and transported preferably at a constant temperature, and should in general not be refrigerated before sale to the final consumer*." In addition, the legislation states that: "*In general, eggs should not be washed or cleaned because such practices can cause damage to the egg shell, which is an effective barrier to bacterial ingress with an array of antimicrobial properties*." However, it is noted that: "*However, egg-washing systems subject to authorisation and operating under carefully controlled conditions are used in some Member States with good results*." In Member States in which packing centres are authorised to wash eggs, the legislation encourages the development of national guides to good practice for egg-washing systems by the food business operators. Fikiin et al. (2020) challenge the current EU regulation and state that it places too great an emphasis on the possibility of eggshell condensation and *Salmonella*-related safety risks, while overlooking other substantial safety and quality hazards; including obscuring or missing temperature and humidity control requirements, which raises concerns about chilled storage while tolerating handling at high temperatures (Fikiin, Akterian, & Stankov, 2020). Under European legislation shelf-life is referred to as the "date of minimum durability". The date of minimum durability of a foodstuff is the date until which the foodstuff retains its specific properties when properly stored. For the sake of clarity, the date of minimum durability for eggs is fixed at not more than 28 days after laying.

The Code of Practice for British Lion eggs mandates vaccination of pullets against SE and ST (BEIC, 2013a). The Code of Practice does not require eggs to be refrigerated at retail. However, the Code of Practice states that throughout the supply chain, eggs should be stored at a constant temperature below 20°C under conditions which prevent surface condensation. The temperature must be recorded during egg storage and delivery. Grade A hen eggs, which are the highest grade and are sold as shell eggs, may not be washed (FSAI, 2010). Only grade B hen eggs may be washed and these cannot be sold at retail, but to other food businesses where they will be processed (FSAI, 2010). British Lion eggs must carry a 'best before' date and the Lion mark on the shell and on the pack. All Lion eggs have a best-before date of no more than 27 days from lay, making them fresher than required by law.

2.4.7 Traceability

Many countries implement legislative requirements on robust through chain traceability. The Codex Alimentarius Commission (CAC) is the body responsible for all matters regarding the implementation of the Joint FAO/WHO Food Standards Programme. The CAC directions set out a one-step forward, one-step back approach: *i.e.*, the previous source where the ingredient/food was obtained/purchased and where the next destination in the supply chain is. This

legislation has been adopted by FSANZ (Standard 3.2.2), the EU (Regulation EC No.178/2002) and the US FDA (CFR Title 21). This approach, however, can be slow and cumbersome and the repercussions can be severe in a food safety breakdown/crisis. To facilitate a more timely response, industry members may adopt an approach of greater visibility throughout the supply chain, by tracing back/tracking forward further than one step. An effective traceability system enables governmental agencies, food producers, food distributors and food retailers to take the necessary action swiftly and effectively to keep the affected food products from endangering consumers, while reducing the costs of food recalls.

A traceability system is a simple process of data collection and recording which can provide evidence of the path a product takes through the supply chain. Identification is critical to a successful traceability program and is usually accomplished by labels. Any number of technologies can be employed for labelling, including barcoding and more sophisticated radio frequency identification (RFID)-based technologies. Traceability systems vary widely in the information they collect as well as on the level of precision with which a product's movement is recorded. A well-designed traceability system can track products at each stage, including harvest, processing, transport, storage, distribution and sales. There have been a number of recent advances in food traceability in terms of technology (Astill et al., 2019; Badia-Melis, Mishra, & Ruiz-García, 2015), which hold the possibility of having the traceability information absolutely centralised across the supply chain and enabling real-time access and action where necessary.

In practice, however, traceability is challenging as the egg supply chain is quite fragmented (Australian Eggs Limited, 2020c). Multiple production methods are undertaken on farms making identifying, separating, segregating and tracing system inputs and outputs complicated. Production and trade records are hard to verify and can easily be manipulated, allowing for potential mislabelling and fraud. In addition, egg farmers operating small enterprises may be daunted by the cost and complexity of establishing an on-farm traceability system.

2.4.7.1 Australia

In Australia, it is compulsory for all commercial egg producers to mark each egg so it can be traced back to its farm of origin. In Australia, only a mark identifying the producer is mandatory. However, a date and/or batch number are recommended to further enhance traceability. A review of egg stamping undertaken by NSW DPI in 2015 concluded that this practice improved traceability across NSW (NSW DPI, 2015). At the time of the review in 2015, it was noted that egg stamping would be essential if locally acquired SE were to emerge in Australia, to limit the extent of SE outbreaks by facilitating rapid trace-back and targeted quarantine measures. Indeed, the value of egg stamping was observed during the 2018 NSW SE outbreak and assisted in subsequent food safety recalls (FSANZ, 2021a). Egg-stamping also helped map the complexity of the supply chain in the SE outbreak (see Table 6).

Eggs can be stamped at the farm where they are produced or at a grading facility. This means that issues may arise with the movement of unstamped, ungraded eggs. For example, when eggs are traded between wholesalers before going to processing or foodservice. This situation also increases the risk of co-mingling. During the NSW SE outbreak investigation three IPs were identified to be moving unstamped, ungraded eggs to other grading facilities, some of which also housed laying hens.

Comprehensive traceability systems are implemented on a voluntary basis and the NSW SE outbreak sparked consideration of how this could be achieved more consistently (Australian Eggs Limited, 2021d). Improvements in traceability options would not only provide benefits for consumers, but also egg farmers who want a greater level of certainty about the origin of eggs they purchase so they can ensure strong biosecurity is maintained across their supply chain (Australian Eggs Limited, 2021d). As part of the review of Standard 4.2.5 (FSANZ, 2021a), FSANZ is considering traceability requirements, including individual identification requirements for eggs (i.e. egg stamping) and new food safety and traceability technologies that have been developed since the standard was introduced.

The recent NSW SE outbreak and foodborne illness data for *Salmonella*-egg related outbreaks including other serovars (Table 5), demonstrates that there is a continued need for effective traceability of eggs. The large number of people affected and hospitalised confirms the importance of improving the speed of traceability. A number of industry and government-funded projects focused on enhancing egg traceability are currently underway in Australia.

Australian Eggs has undertaken a project to help egg farmers to adopt on-farm traceability (Australian Eggs Limited, 2021h). The project will span from 2020-2023 and will involve the creation and extension of new resources to help farmers implement on-farm traceability systems to suit their business. As part of this project, the software tool EggTrace was developed. EggTrace was created with a focus on small egg farmers, who may not have any traceability systems in place. EggTrace enables every farm to capture traceability information digitally and store all important traceability records in one place. In the event of a recall or food safety issue, EggTrace provides the transparency required to act quickly.

GS1 and the Commonwealth Scientific and Industrial Research Organisation (CSIRO) have undertaken a project to review current egg traceability systems and available technologies (Australian Eggs Limited, 2020c). The project aims to define available technologies and methods that could be adopted to support enhanced traceability in the Australian egg industry.

2.4.7.2 Traceability in other countries

In Europe, the only requirement for egg marking is that the producer code is stamped on the egg at the production site. When eggs are delivered directly to the food industry for processing and there is sufficient guarantee of their final destination, member states may grant exemptions from the egg marking requirement to operators who so request. Labelling requirements are also in place for the packaging of Class A and Class B eggs. Packaging of Class A eggs must include the packing centre code, quality grading (*i.e.* Class A), weight grading, date of minimum durability, the wording ‘washed eggs’ for eggs washed in accordance with regulations, storage requirements and farming method (*e.g.* organic). Packaging of Class B eggs must include the packing centre code, quality grading (*i.e.* Class B) and the packing date. For loose egg sales (i.e. no packaging), the following information must be given; quality grading, weight grading, farming method, an explanation of the meaning of the producer code and the date of minimum durability.

In the UK, the British Lion eggs scheme requires full traceability of hens, eggs and feed. Lion eggs are marked on farm with the producer establishment number, which shows the system of production (organic, free range, barn or caged), country of origin and the farm where the eggs were laid (for a summary see (BEIC, 2013b)). A website – www.lioneggfarms.co.uk – also allows consumers to trace British Lion eggs back to the farm from the code on their eggs. To guarantee traceability, all breeding farms, hatcheries, rearing and laying farms, feed mills and packing centres involved in the production of British Lion eggs must be approved and registered by BEIC, which maintains a ‘live’ database of all Lion premises. All British Lion pullet rearing and laying flocks must be accompanied by a unique passport certificate and all British Lion egg movement has to be fully traceable. All Lion sites have to keep full records for two years of all monitoring and testing procedures, including audits.

In the USA, the FDA has a new proposed rule “Requirements for Additional Traceability Records for Certain Foods” (Food Traceability Proposed Rule) which the Agency announced on September 21, 2020 (FDA, 2020b). The proposed rule is a key component of the FDA’s New Era of Smarter Food Safety Blueprint (FDA, 2020a), of which a core element is tech-enabled traceability and the goal of end-to-end traceability throughout the food safety system. The proposed rule places additional traceability recordkeeping requirements (beyond what is already required in existing regulations) for persons who manufacture, process, pack, or hold foods the FDA has designated for inclusion on the Food Traceability List (FTL) (FDA, 2021b). Shell eggs of domesticated chickens are on the FTL. The proposed rule would exempt shell egg producers with fewer than 3,000 laying hens on farm and those farms that sell directly to consumers (e.g. sales at farmer’s markets, roadside stands, over the internet etc). Exemptions are also proposed for

eggs when all eggs produced at the particular farm receive a treatment (as outlined in the Egg Safety Rule). These exemptions would apply to the food throughout the supply chain, including before the processing is performed. At the core of this proposal is a requirement for those who manufacture, process, pack or hold foods on the FTL to establish and maintain records containing Key Data Elements (KDEs) associated with different Critical Tracking Events (CTEs). The proposed rule identifies growing, receiving, transforming, creating, and shipping as the CTEs for which records containing KDEs would be required. The KDEs required would vary depending on the CTE that is being performed (FDA, 2020c). The records required at each CTE would need to contain and link the traceability lot code of the food to the relevant KDEs. The proposed rule would also require anyone subject to the rule to establish and maintain traceability program records, that help regulators understand an entity's traceability program (i.e. information needed to understand data provided within the required records). The proposed rule would also require that: (i) records be maintained as either original paper records, electronic records, or true copies, (ii) traceability records be provided to FDA as soon as possible but no later than 24 hours after a request is made and, (iii) an electronic sortable spreadsheet containing relevant traceability information be provided to FDA within 24 hours of a request when necessary to assist FDA during an outbreak, recall or other threat to public health.

3. Conclusion

In NSW, a broad range of interventions were put in place to prevent foodborne salmonellosis following the release of the new NSW Food Safety Strategy 2015–2021. Though the incidence of foodborne salmonellosis has declined in this period, the recent emergence of SE has raised questions about the ability of current compliance requirements for Standard 4.2.5 to adequately manage risks. As SE can be highly persistent in both infected birds and diverse environmental reservoirs, complete eradication of SE from the Australian egg production environment is challenging. It is prudent to view SE as part of the broad ecosystem and therefore a continuing threat to commercial egg production. The NSW SE outbreak investigation also highlighted the complex network of movement of people, vehicles and equipment between premises and the time lag between introduction of SE into a premise and its establishment and detection on farm. Continued vigilance and maintenance of rigorous biosecurity practices within and between premises and strict hygiene procedures will be crucial for the Australian egg industry into the future. Currently, there are no live SE vaccines available in Australia and only partial protection against the Australian SE 7A strain was reported to be provided by the live attenuated Vaxsafe ST (Bioproperties®) vaccine (Clark et al., 2021). Therefore, there is a strong emphasis on the implementation of other risk reduction practices along the entire egg production continuum; from the breeding, hatching, pullet rearing and egg laying environment. In addition, pathways for contamination of eggs by SE can be influenced by storage, handling and food preparation practices.

A Control Order in NSW is currently in effect until the 30th of June 2024 and establishes minimum biosecurity standards and mandatory testing requirements for the poultry and egg industries. The Control Order is legally enforceable under the *Biosecurity Act 2015* (NSW). At the time of writing, NSW is still in management mode and aiding in the decontamination and clearance of SE from IPs.

A national approach to manage SE will be determined by FSANZ through their review of Standard 4.2.5. A range of control measures have been identified by FSANZ (2021a) for consideration, including biosecurity measures, flock testing, temperature control for intact shell eggs and traceability. Risk reduction practices for SE are inter-dependent, as currently there is no single control measure which ensures full protection against SE. Continued collection of information on SE prevalence in the egg-laying environment will be vital to provide a baseline for assessing the effectiveness and appropriateness of any implemented SE risk reduction strategies. This information will also provide a baseline for monitoring future impacts of industry changes in composition and activity on microbial risks. In Australia, major retailers are committed to phasing out cage eggs by 2023 or 2025 (Australian Eggs Limited, 2019). There is conflicting evidence on the effect of different housing systems as a risk factor for *Salmonella* (Sodagari et al., 2020). As the industry continues to change from caged houses to cage-free, pasture, or free range, these risk factors and the

sampling methodologies employed will need to be evaluated. It must be emphasised that while this risk assessment has largely focused on the control of SE in layer flocks, other serotypes cannot be neglected. As highlighted in their scientific opinion on *Salmonella* control in poultry flocks and its public health impact, EFSA (2019) recommended that reporting of all individual *Salmonella* serovars in poultry flocks would facilitate source attribution and epidemiological studies. A broader awareness of the prevalence of all *Salmonella* serovars within the poultry industry, would also provide an early warning on whether focusing on control programs that target a limited range of serovars is creating a niche for expansion of other serovars.

Finally, in conjunction with those measures described on-farm and at retail, the prevention of food safety events in humans depends on subsequent egg handling and food preparation practices. There have been a number of recent studies on risk factors in consumer egg handling practices, from egg procurement to final consumption at home (Cardoso et al., 2021; Junqueira et al., 2022). The consumption of raw and undercooked eggs and egg products has largely driven outbreaks of foodborne illness in countries in which SE has become established in commercial egg laying flocks. To reduce risk, it has been recommended that consumers acquire eggs with a lower probability of being contaminated with *Salmonella* (i.e. those under an official entity or producer guarantee of being *Salmonella*-free). However, eggs with a *Salmonella*-free guarantee are only available in a few countries. The vast majority of egg-related outbreaks in NSW are due to ingestion of "*raw*" or "*undercooked*" eggs or egg products. In an Australian study exploring people's egg handling and cooking practices and their knowledge and attitudes regarding the associated food safety risks, it was identified that there is an underestimation of "risky behaviour" (Whiley, Clarke, & Ross, 2017). For example, Whiley et al. (2017) reported that 84% of survey participants indicated that they did not consume raw eggs, but subsequently 86% indicated that they had eaten mixture/batter containing raw eggs. A recent survey of NSW consumers revealed that a number of egg handling practices were routinely undertaken that can increase the risk of foodborne illness (NSW Food Authority, 2020e). For example, of those NSW consumers surveyed, 43% did not know that they should always discard eggs that have dirty or cracked shells (NSW Food Authority, 2020e). In addition, 40% of the NSW consumers surveyed stated that they *do not* store eggs in an egg carton in the fridge and 55% *did not know* that they should store eggs in an egg carton in the fridge (NSW Food Authority, 2020e). To reduce the risk of *Salmonella* infection from eggs at home, consumers are advised to follow the NSW Food Authority's recommendations on the safe handling of eggs (NSW Food Authority, 2021c).

References

Abbas, A., Lichtman, A., & Pillai, S. (2015). *Cellular and Molecular Immunology* (8th Edition ed.): Elsevier.

ABS. (2020a). Apparent Consumption of Selected Foodstuffs, Australia. Retrieved from https://www.abs.gov.au/statistics/health/health-conditions-and-risks/apparent-consumption-selected-foodstuffs-australia/latest-release#data-download

ABS. (2020b). Apparent Consumption of Selected Foodstuffs, Australia methodology. Retrieved from https://www.abs.gov.au/methodologies/apparent-consumption-selected-foodstuffs-australia-methodology/2019-20

Aehle, S., & Curtiss, R. (2017). Chapter 14 - Current and Future Perspectives on Development of *Salmonella* Vaccine Technologies. In S. C. Ricke & R. K. Gast (Eds.), *Producing Safe Eggs* (pp. 281-299). San Diego: Academic Press.

Ahaduzzaman, M., Groves, P. J., Walkden-Brown, S. W., & Gerber, P. F. (2021). A molecular based method for rapid detection of *Salmonella* spp. in poultry dust samples. *MethodsX, 8*, 101356. doi:10.1016/j.mex.2021.101356

Animal Health Australia. (2020). National Farm Biosecurity Technical Manual for Egg Production. Retrieved from https://www.australianeggs.org.au/assets/dms-documents/FINAL_National-Farm-Biosecurity-Technical-Manual-for-Egg-Production-September-2020-v2.pdf

Animal Health Australia. (2021). National *Salmonella* Enteritidis Response Management Plan. Retrieved from https://animalhealthaustralia.com.au/industry-publications/

APVMA. (2017). *Antibiotic resistance in animals*. Retrieved from https://apvma.gov.au/sites/default/files/publication/27326-final_amr_report-_for_publishing_v02_140817_a939399.pdf

APVMA. (2021). APVMA guideline for autogenous vaccine permits. Retrieved from https://apvma.gov.au/node/1108

Arnold, M. E., Martelli, F., McLaren, I., & Davies, R. H. (2014). Estimation of the sensitivity of environmental sampling for detection of *Salmonella* in commercial layer flocks post-introduction of national control programmes. *Epidemiology and infection, 142*(5), 1061-1069. doi:10.1017/S0950268813002173

ASTAG on AMR. (2018). Importance Ratings and Summary of Antibacterial Uses in Human and Animal Health in Australia. Retrieved from https://www.amr.gov.au/resources/importance-ratings-and-summary-antibacterial-uses-human-and-animal-health-australia

Astill, J., Dara, R. A., Campbell, M., Farber, J. M., Fraser, E. D. G., Sharif, S., & Yada, R. Y. (2019). Transparency in food supply chains: A review of enabling technology solutions. *Trends in Food Science and Technology, 91*, 240-247. doi:10.1016/j.tifs.2019.07.024

Australian Eggs Limited. (2019). *ANNUAL REPORT 2018/19*. Retrieved from https://www.australianeggs.org.au/who-we-are/annual-reports

Australian Eggs Limited. (2020a). Annual Report 2020. Retrieved from https://www.australianeggs.org.au/who-we-are/annual-reports

Australian Eggs Limited. (2020b). *Salmonella* Enteritidis Operational Response Plan. Retrieved from https://www.australianeggs.org.au/dms/5002-Salmonella-Enteritidis-Operational-Response-Plan.pdf

Australian Eggs Limited. (2020c). Traceability Technology Desktop Review. Retrieved from https://www.australianeggs.org.au/what-we-do/leading-research/traceability-technology-desktop-review

Australian Eggs Limited. (2021a). Australian Egg Industry Overview. Retrieved from https://www.australianeggs.org.au/egg-industry

Australian Eggs Limited. (2021b). Best practice for on-farm euthanasia of spent layer hens. Retrieved from https://www.australianeggs.org.au/what-we-do/leading-research/best-practice-for-on-farm-euthanasia-of-spent-layer-hens

Australian Eggs Limited. (2021c). Grading, Washing & Packing. Retrieved from https://www.australianeggs.org.au/for-farmers/tools-and-training/salmonella-risk-assessment-toolkit/grading-washing-and-packing#faq-269

Australian Eggs Limited. (2021d). Health and disease. Retrieved from https://www.australianeggs.org.au/what-we-do/health-and-disease

Australian Eggs Limited. (2021e). Post-Grading Processes. Retrieved from https://www.australianeggs.org.au/for-farmers/tools-and-training/salmonella-risk-assessment-toolkit/post-grading-processes

Australian Eggs Limited. (2021f). *Salmonella* Enteritidis A GUIDE FOR PRODUCERS. Retrieved from https://www.australianeggs.org.au/assets/dms-documents/SE-guide-for-producers.pdf

Australian Eggs Limited. (2021g). *Salmonella* RISK ASSESSMENT TOOLKIT. Retrieved from https://www.australianeggs.org.au/for-farmers/tools-and-training/salmonella-risk-assessment-toolkit

Australian Eggs Limited. (2021h). Traceability. Retrieved from https://www.australianeggs.org.au/for-farmers/traceability

Australian Eggs Limited. (2022). How Do Chickens Make Eggs? Retrieved from https://www.australianeggs.org.au/farming/how-chickens-make-eggs

Badia-Melis, R., Mishra, P., & Ruiz-García, L. (2015). Food traceability: New trends and recent advances. A review. *Food Control, 57*, 393-401. doi:10.1016/j.foodcont.2015.05.005

BEIC. (2013a). Code of Practice for Lion eggs, The British Egg Industry Council. Retrieved from http://www.britisheggindustrycouncil.co.uk/download/LCoPV7.pdf

BEIC. (2013b). Lion Code of Practice Summary. Retrieved from https://www.egginfo.co.uk/sites/default/files/Lion%20Code%20of%20Practice%20leaflet.pdf

BEIC. (2021). British Lion Code of Practice. Retrieved from http://www.britisheggindustrycouncil.co.uk/british-lion-code-of-practice/

Braden, C. R. (2006). *Salmonella enterica* Serotype Enteritidis and Eggs: A National Epidemic in the United States. *Clinical Infectious Diseases, 43*(4), 512-517. doi:10.1086/505973

British Lion eggs. (2021a). British Lion eggs. Retrieved from https://www.egginfo.co.uk/british-lion-eggs#:~:text=British%20Lion%20eggs%20The%20British%20Lion%20is%20the,have%20been%20sold%20since%20its%20launch%20in%201998.

British Lion Eggs. (2021b). A great British success story. Retrieved from https://www.egginfo.co.uk/british-lion-eggs/about/success-story

British Lion Eggs. (2021c). Industry data. Retrieved from https://www.egginfo.co.uk/egg-facts-and-figures/industry-information/data

Brochu, N. M., Guerin, M. T., Varga, C., Lillie, B. N., Brash, M. L., & Susta, L. (2021). Demographic Characteristics and Husbandry and Biosecurity Practices of Small Poultry Flocks in Ontario, Canada. *Avian Diseases, 65*(2), 287-294, 288.

Brown, A. (2020). Coronavirus: Poultry breeders see surge in demand for backyard chicken. Retrieved from https://www.canberratimes.com.au/story/6713333/poultry-breeders-see-egg-streme-demand-for-backyard-chickens/

Cardoso, M. J., Nicolau, A. I., Borda, D., Nielsen, L., Maia, R. L., Møretrø, T., . . . Teixeira, P. (2021). *Salmonella* in eggs: From shopping to consumption—A review providing an evidence-based analysis of risk factors. *Comprehensive Reviews in Food Science and Food Safety, 20*(3), 2716-2741. doi:10.1111/1541-4337.12753

Carrique-Mas, J. J., & Davies, R. H. (2008). Bacteriological detection of *Salmonella* Enteritidis in eggs: a review. *Revue scientifique et technique (International Office of Epizootics), 27*(3), 657-664.

Carrique-Mas, J. J., Marín, C., Breslin, M., McLaren, I., & Davies, R. (2009). A comparison of the efficacy of cleaning and disinfection methods in eliminating *Salmonella* spp. from commercial egg laying houses. *Avian Pathology, 38*(5), 419-424. doi:10.1080/03079450903193768

CDC. (2016). Multistate Outbreak of *Salmonella* Oranienburg Infections Linked to Good Earth Egg Company Shell Eggs (Final Update). Retrieved from https://www.cdc.gov/salmonella/oranienburg-10-16/index.html

CDC. (2018a). National Enteric Disease Surveillance: *Salmonella* Annual Report, 2016. Retrieved from https://www.cdc.gov/nationalsurveillance/pdfs/2016-Salmonella-report-508.pdf

CDC. (2018b). Outbreak of *Salmonella* Infections Linked to Gravel Ridge Farms Shell Eggs - Final Update. Retrieved from https://www.cdc.gov/salmonella/enteritidis-09-18/index.html

CDC. (2018c). Understanding How Vaccines Work. Retrieved from https://www.cdc.gov/vaccines/hcp/conversations/understanding-vacc-work.html

Chen, J., Thesmar, H. S., & Kerr, W. L. (2005). Outgrowth of Salmonellae and the Physical Property of Albumen and Vitelline Membrane as Influenced by Egg Storage Conditions. *Journal of Food Protection, 68*(12), 2553-2558. doi:10.4315/0362-028x-68.12.2553

Chousalkar, K. K., Sexton, M., McWhorter, A., Hewson, K., Martin, G., Shadbolt, C., & Goldsmith, P. (2017). *Salmonella typhimurium* in the Australian egg industry: Multidisciplinary approach to addressing the public health challenge and future directions. *Critical Reviews in Food Science and Nutrition, 57*(12), 2706-2711. doi:10.1080/10408398.2015.1113928

Clark, C., Collins, A., Gao, Y., Underwood, G., Jackson, C., Williamson, S., . . . Groves, P. (2021). *POTENTIAL OF A LIVE SALMONELLA TYPHIMURIUM VACCINE TO PROVIDE CROSSPROTECTION AGAINST THE NSW SALMONELLA ENTERITIDIS STRAIN IN LAYERS.* Paper presented at the 2021 AVPA Virtual Scientific Meeting.

Clay, C. E., & Board, R. G. (1991). Growth of *Salmonella* enteritidis in artificially contaminated hens' shell eggs. *Epidemiology and infection, 106*(2), 271-281. doi:10.1017/s095026880004841x

Clem, A. S. (2011). Fundamentals of vaccine immunology. *Journal of global infectious diseases, 3*(1), 73-78. doi:10.4103/0974-777X.77299

Cogan, T. A., Jørgensen, F., Lappin-Scott, H. M., Benson, C. E., Woodward, M. J., & Humphrey, T. J. (2004). Flagella and curli fimbriae are important for the growth of *Salmonella enterica* serovars in hen eggs. *Microbiology (Reading), 150*(Pt 4), 1063-1071. doi:10.1099/mic.0.26791-0

Communicable Diseases Branch. (2014). *NSW OzFoodNet Annual Surveillance Report: 2013*. Sydney: Health Protection NSW Retrieved from https://www.health.nsw.gov.au/Infectious/foodborne/Publications/nsw-ofn-annual-report-2013.pdf

Communicable Diseases Branch. (2015). *NSW OzFoodNet Annual Surveillance Report: 2014*. Sydney: Health Protection NSW Retrieved from https://www.health.nsw.gov.au/Infectious/foodborne/Publications/nsw-ofn-annual-report-2014.pdf

Communicable Diseases Branch. (2016). *NSW OzFoodNet Annual Surveillance Report: 2015*. Sydney: Health Protection NSW Retrieved from https://www.health.nsw.gov.au/Infectious/foodborne/Publications/nsw-ofn-annual-report-2015.pdf

Communicable Diseases Branch. (2017). *NSW OzFoodNet Annual Surveillance Report: 2016*. Sydney: Health Protection NSW Retrieved from https://www.health.nsw.gov.au/Infectious/foodborne/Publications/nsw-ofn-annual-report-2016.pdf

Communicable Diseases Branch. (2018). *NSW OzFoodNet Annual Surveillance Report: 2017*. Sydney: Health Protection NSW Retrieved from https://www.health.nsw.gov.au/Infectious/foodborne/Publications/nsw-ofn-annual-report-2017.pdf

Communicable Diseases Branch. (2019). *NSW OzFoodNet Annual Surveillance Report: 2018*. Sydney: Health Protection NSW Retrieved from https://www.health.nsw.gov.au/Infectious/foodborne/Publications/nsw-ofn-annual-report-2018.pdf

Communicable Diseases Branch. (*In print-a*). *NSW OzFoodNet Annual Surveillance Report: 2019*. Sydney: Health Protection NSW

Communicable Diseases Branch. (*In print-b*). *NSW OzFoodNet Annual Surveillance Report: 2020*. Sydney: Health Protection NSW

Correia-Gomes, C., & Sparks, N. (2020). Exploring the attitudes of backyard poultry keepers to health and biosecurity. *Preventive Veterinary Medicine, 174*, 104812. doi:10.1016/j.prevetmed.2019.104812

Cuttell, L., Groves, M., & Wilson, A. (2014). *2014 Microbiological baseline survey of the Queensland egg production environment*. Retrieved from http://esvc000111.wic055u.server-web.com/images/PDF/egg_egg_products/2014%20microbiological%20survey%20of%20queensland%20egg%20farms.pdf

DAFF. (2009). National Farm Biosecurity Manual, Poultry Production. Retrieved from https://www.awe.gov.au/sites/default/files/sitecollectiondocuments/animal-plant/pests-diseases/biosecurity/poultry-bio-manual/poultry-biosecurity-manual.pdf

Dallman, T., Inns, T., Jombart, T., Ashton, P., Loman, N., Chatt, C., . . . Cleary, P. (2016). Phylogenetic structure of European *Salmonella* Enteritidis outbreak correlates with national and international egg distribution network. *Microbial genomics, 2*(8), e000070. doi:10.1099/mgen.0.000070

Davies, R., & Breslin, M. (2004). Observations on *Salmonella* contamination of eggs from infected commercial laying flocks where vaccination for *Salmonella enterica* serovar Enteritidis had been used. *Avian Pathology, 33*(2), 133-144. doi:10.1080/03079450310001652040

Davies, R. H., & Breslin, M. (2003). Persistence of *Salmonella* Enteritidis Phage Type 4 in the environment and arthropod vectors on an empty free-range chicken farm. *Environmental Microbiology, 5*(2), 79-84. doi:10.1046/j.1462-2920.2003.00387.x

Davies, R. H., & Wray, C. (1995). Mice as carriers of *Salmonella* Enteritidis on persistently infected poultry units. *The Veterinary record, 137*(14), 337-341. doi:10.1136/vr.137.14.337

Davies, R. H., & Wray, C. (1996). Persistence of *Salmonella* Enteritidis in poultry units and poultry food. *British Poultry Science, 37*(3), 589-596. doi:10.1080/00071669608417889

DAWE. (2019). Bird biosecurity. Retrieved from https://www.awe.gov.au/biosecurity-trade/pests-diseases-weeds/protect-animal-plant/bird-owners

DAWE. (2020a). 148-2020 – Notice of the department's assessment of an application to import live attenuated *Salmonella* vaccines. Retrieved from https://www.awe.gov.au/biosecurity-trade/import/industry-advice/2020/148-2020

DAWE. (2020b). *National Residue Survey 2019–20 Annual Summary*. Retrieved from https://www.agriculture.gov.au/sites/default/files/documents/nrs-2019-20-summary.pdf

DAWE. (2021). National Residue Survey. Retrieved from https://www.agriculture.gov.au/ag-farm-food/food/nrs

DAWR. (2021). National *Salmonella* Enteritidis Monitoring & Accreditation Program Guidelines. Retrieved from https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0020/722081/NSEMAP-Guidelines.pdf

De Reu, K., Grijspeerdt, K., Messens, W., Heyndrickx, M., Uyttendaele, M., Debevere, J., & Herman, L. (2006). Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including *Salmonella enteritidis*. *International Journal of Food Microbiology, 112*(3), 253-260. doi:10.1016/j.ijfoodmicro.2006.04.011

Dewaele, I., Van Meirhaeghe, H., Rasschaert, G., Vanrobaeys, M., De Graef, E., Herman, L., . . . De Reu, K. (2012). Persistent *Salmonella* Enteritidis environmental contamination on layer farms in the context of an implemented national control program with obligatory vaccination. *Poultry Science, 91*(2), 282-291. doi:10.3382/ps.2011-01673

DeWinter, L. M., Ross, W. H., Couture, H., & Farber, J. F. (2011). Risk Assessment of Shell Eggs Internally Contaminated with *Salmonella* Enteritidis. *International Food Risk Analysis Journal, 1*(1), 40-81.

DoH. (2018). *Review of published and grey literature on the presence of antimicrobial resistance in food in Australia and New Zealand*. Retrieved from https://www.amr.gov.au/resources/review-published-and-grey-literature-presence-antimicrobial-resistance-food-australia-and

DoH. (2020). *Australia's National Antimicrobial Resistance Strategy - 2020 and Beyond*. Retrieved from https://www.amr.gov.au/resources/australias-national-antimicrobial-resistance-strategy-2020-and-beyond

DoH, & DAWE. (2020). AMR and animal health in Australia. Retrieved from https://www.amr.gov.au/about-amr/amr-australia/amr-and-animal-health-australia

Ducatelle, R., & Van Immerseel, F. (2011). 8 - Management and sanitation procedures to control *Salmonella* in laying hen flocks. In F. Van Immerseel, Y. Nys, & M. Bain (Eds.), *Improving the Safety and Quality of Eggs and Egg Products* (pp. 146-162): Woodhead Publishing.

EFSA Panel on Biological Hazards, Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., . . . Davies, R. (2019). *Salmonella* control in poultry flocks and its public health impact. *EFSA Journal, 17*(2), e05596. doi:10.2903/j.efsa.2019.5596

Elanco. (2021). Poultry Vaccines. Retrieved from https://www.elanco.us/poultry-vaccines

EU. (2005). COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs.

EU. (2010). COMMISSION REGULATION (EU) No 200/2010 of 10 March 2010 implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards a Union target for the reduction of the prevalence of *Salmonella* serotypes in adult breeding flocks of *Gallus gallus*.

EU. (2011). COMMISSION REGULATION (EU) No 517/2011 of 25 May 2011 implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards a Union target for the reduction of the prevalence of certain *Salmonella* serotypes in laying hens of *Gallus gallus* and amending Regulation (EC) No 2160/2003 and Commission Regulation (EU) No 200/2010.

FDA. (2009). 21 CFR Parts 16 and 118 Prevention of *Salmonella* Enteritidis in Shell Eggs During Production, Storage, and Transportation; Final Rule.

FDA. (2016). FDA Investigates Outbreak of *Salmonella* Oranienburg Linked to Shell Eggs. Retrieved from https://www.fda.gov/food/outbreaks-foodborne-illness/fda-investigates-outbreak-salmonella-oranienburg-linked-shell-eggs

FDA. (2017). Environmental Sampling and Detection of *Salmonella* in Poultry Houses. Retrieved from https://www.fda.gov/food/laboratory-methods-food/environmental-sampling-and-detection-salmonella-poultry-houses#:~:text=Environmental%20Sampling%20and%20Detection%20of%20Salmonella%20in%20Poultry,%207.5%20g%20%208%20more%20rows%20

FDA. (2020a). NEW ERA OF SMARTER FOOD SAFETY, FDA's Blueprint for the Future. Retrieved from https://www.fda.gov/media/139868/download

FDA. (2020b). Pre-recorded Webinar to Discuss Food Traceability Proposed Rule. Retrieved from https://www.fda.gov/food/food-safety-modernization-act-fsma/pre-recorded-webinar-discuss-food-traceability-proposed-rule

FDA. (2020c). Which Key Data Elements Would Apply to Me? Retrieved from https://www.fda.gov/food/food-safety-modernization-act-fsma/which-key-data-elements-would-apply-me

FDA. (2021a). 21 CFR Part 115.50 Refrigeration of shell eggs held for retail distribution.

FDA. (2021b). FSMA Proposed Rule for Food Traceability. Retrieved from https://www.fda.gov/food/food-safety-modernization-act-fsma/fsma-proposed-rule-food-traceability

PART 118 - PRODUCTION, STORAGE, AND TRANSPORTATION OF SHELL EGGS, (2021c).

FDA. (2021d). What You Need to Know About Egg Safety. Retrieved from https://www.fda.gov/food/buy-store-serve-safe-food/what-you-need-know-about-egg-safety

Fikiin, K., Akterian, S., & Stankov, B. (2020). Do raw eggs need to be refrigerated along the food chain?: *Is the current EU regulation ensuring high-quality shell eggs for the European consumers? Trends in Food Science and Technology, 100*, 359-362. doi:10.1016/j.tifs.2020.04.003

Food Safety Information Council. (2020). National warning on backyard chooks after *Salmonella* outbreaks (9 July 2020). Retrieved from https://foodsafety.asn.au/topic/national-warning-on-backyard-chooks-after-salmonella-outbreaks-9-july-2020/

Ford, L., Carter, G. P., Wang, Q., Seemann, T., Sintchenko, V., Glass, K., . . . Kirk, M. D. (2018). Incorporating Whole-Genome Sequencing into Public Health Surveillance: Lessons from Prospective Sequencing of *Salmonella* Typhimurium in Australia. *Foodborne Pathogens and Disease, 15*(3), 161-167. doi:10.1089/fpd.2017.2352

Ford, L., Glass, K., Veitch, M., Wardell, R., Polkinghorne, B., Dobbins, T., . . . Kirk, M. D. (2016). Increasing Incidence of *Salmonella* in Australia, 2000-2013. *PLoS One, 11*(10), e0163989. doi:10.1371/journal.pone.0163989

FSA. (2019). FSA issues precautionary egg safety and handling advice concerning one flock code. Retrieved from https://www.food.gov.uk/news-alerts/alert/fsa-fafa-02-2019

FSA. (2020). FSA issues precautionary safety and handling advice for specific eggs. Retrieved from https://www.food.gov.uk/news-alerts/news/fsa-issues-precautionary-safety-and-handling-advice-for-specific-eggs

FSAI. (2010). Egg washing. Retrieved from https://www.fsai.ie/faq/egg_washing.html

FSANZ. (2009a). Public health and safety of eggs and egg products in Australia, Explanatory summary of the risk assessment. Retrieved from https://www.foodstandards.gov.au/publications/documents/Eggs_healthandsafety.pdf

FSANZ. (2009b). SD1, PROPOSAL P301, PRIMARY PRODUCTION AND PROCESSING STANDARD FOR EGGS & EGG PRODUCTS, RISK ASSESSMENT OF EGGS AND EGG PRODUCTS. Retrieved from https://www.foodstandards.gov.au/code/proposals/documents/P301%20Eggs%20PPPS%20DAR%20SD1%20Risk%20Assessment.pdf

FSANZ. (2011). In May 2011 a newspaper article made some claims about the egg standard. Retrieved from https://www.foodstandards.gov.au/code/primaryproduction/egg/pages/inmay2011anewspapera5170.aspx

FSANZ. (2013). *Salmonella* (non-typhoidal). Retrieved from https://www.foodstandards.gov.au/publications/Documents/Salmonella%20(non-typhoidal).pdf

FSANZ. (2018). Food recalls. Retrieved from https://www.foodstandards.gov.au/industry/foodrecalls/Pages/default.aspx

FSANZ. (2019a). *25th Australian Total Diet Study*. Retrieved from https://www.foodstandards.gov.au/publications/Documents/25thAustralianTotalDietStudy.pdf

FSANZ. (2019b). *25th Australian Total Diet Study: Appendices*. Retrieved from https://www.foodstandards.gov.au/publications/Documents/25th%20Australian%20Total%20Diet%20Study%20appendices.pdf

FSANZ. (2019c). *25th Australian Total Diet Study: Appendices*. Retrieved from https://www.foodstandards.gov.au/publications/Documents/25th%20Australian%20Total%20Diet%20Study%20appendices.pdf

FSANZ. (2019d). Food Standards Code. Retrieved from https://www.foodstandards.gov.au/code/Pages/default.aspx

FSANZ. (2019e). Report on Emerging and Ongoing Issues - Annual Report 2019. Retrieved from https://www.foodstandards.gov.au/publications/Pages/Report-on-Emerging-and-Ongoing-Issues-Annual-Report-2019.aspx

FSANZ. (2021a). Call for Information: review of Standard 4.2.5 – Primary production and processing standard for eggs and egg product (Australia only). Retrieved from https://www.foodstandards.gov.au/industry/safetystandards/Documents/W1138%20Egg%20Standard%20Review%20-%20Public%20Information%20Paper.pdf

FSANZ. (2021b). Current food recalls. Retrieved from https://www.foodstandards.gov.au/industry/foodrecalls/recalls/Pages/default.aspx

Gaffney, A., & Kinninment, M. (2020). Backyard chickens in huge demand as coronavirus shopping frenzy empties supermarkets. Retrieved from https://www.abc.net.au/news/2020-03-19/backyard-chickens-in-demand-due-to-coronavirus/12068392

Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Gast, R., Humphrey, T. J., & Van Immerseel, F. (2009). Mechanisms of egg contamination by *Salmonella* Enteritidis. *FEMS Microbiology Reviews, 33*(4), 718-738. doi:10.1111/j.1574-6976.2008.00161.x

Gast, R. K. (2007). Serotype-specific and serotype-independent strategies for preharvest control of food-borne *Salmonella* in poultry. *Avian Diseases, 51*(4), 817-828. doi:10.1637/8090-081807.1

Gast, R. K. (2017). Chapter 2 - Microbiology of Shell Egg Production in the United States. In S. C. Ricke & R. K. Gast (Eds.), *Producing Safe Eggs* (pp. 25-44). San Diego: Academic Press.

Gast, R. K., Guraya, R., Guard-Bouldin, J., & Holt, P. S. (2007). In Vitro Penetration of Egg Yolks by *Salmonella* Enteritidis and *Salmonella* Heidelberg Strains During Thirty-Six-Hour Ambient Temperature Storage. *Poultry Science, 86*(7), 1431-1435. doi:10.1093/ps/86.7.1431

Gast, R. K., Guraya, R., Guard-Bouldin, J., & Holt, P. S. (2008). Multiplication of *Salmonella* Enteritidis on the yolk membrane and penetration to the yolk contents at 30°C in an in vitro egg contamination model. *Journal of Food Protection, 71*(9), 1905-1909. doi:10.4315/0362-028x-71.9.1905

Gast, R. K., & Holt, P. S. (2000). Deposition of Phage Type 4 and 13a Salmonella enteritidis Strains in the Yolk and Albumen of Eggs Laid by Experimentally Infected Hens. *Avian Diseases, 44*(3), 706-710. doi:10.2307/1593116

Gast, R. K., & Jones, D. R. (2017). Chapter 48 - *Salmonella* and Impact on Egg Production. In P. Y. Hester (Ed.), *Egg Innovations and Strategies for Improvements* (pp. 515-521). San Diego: Academic Press.

Gast, R. K., Regmi, P., Guraya, R., Jones, D. R., Anderson, K. E., & Karcher, D. M. (2019). Contamination of eggs by *Salmonella* Enteritidis in experimentally infected laying hens of four commercial genetic lines in conventional cages and enriched colony housing. *Poultry Science, 98*(10), 5023-5027. doi:10.3382/ps/pez222

Gole, V. C., Caraguel, C. G. B., Sexton, M., Fowler, C., & Chousalkar, K. K. (2014). Shedding of *Salmonella* in single age caged commercial layer flock at an early stage of lay. *International Journal of Food Microbiology, 189*, 61-66. doi:10.1016/j.ijfoodmicro.2014.07.030

Gole, V. C., Chousalkar, K. K., Roberts, J. R., Sexton, M., May, D., Tan, J., & Kiermeier, A. (2014). Effect of Egg Washing and Correlation between Eggshell Characteristics and Egg Penetration by Various *Salmonella* Typhimurium Strains. *PLoS One, 9*(3), e90987. doi:10.1371/journal.pone.0090987

Gole, V. C., Torok, V., Sexton, M., Caraguel, C. G. B., & Chousalkar, K. K. (2014). Association between indoor environmental contamination by *Salmonella enterica* and contamination of eggs on layer farms. *Journal of clinical microbiology, 52*(9), 3250-3258. doi:10.1128/jcm.00816-14

Graham, D., Li, W., & Hartmann, Z. (2021). Sizing of the backyard and micro-commercial egg production in Australia. Retrieved from https://www.australianeggs.org.au/assets/Sizing-of-the-backyard-and-micro-commercial-egg-production-in-Australia-Sapere-2021.pdf

Grimes, T., & Jackson, C. (2015). Code of practice for biosecurity in the egg industry, Second Edition. Retrieved from https://www.australianeggs.org.au/dmsdocument/719-biosecurity-code-of-practice-2nd-edition

Hara-Kudo, Y., Sakakibara, Y., Konuma, H., Sawada, T., & Kumagai, S. (2001). Laying season and egg shell cracks on the growth of *Salmonella* Enteritidis in the egg albumen during storage. *Journal of Food Protection, 64*(8), 1134-1137. doi:10.4315/0362-028x-64.8.1134

Hennessy, T. W., Hedberg, C. W., Slutsker, L., White, K. E., Besser-Wiek, J. M., Moen, M. E., . . . Osterholm, M. T. (1996). A NATIONAL OUTBREAK OF *SALMONELLA ENTERITIDIS* INFECTIONS FROM ICE CREAM. *New England Journal of Medicine, 334*(20), 1281-1286. doi:10.1056/nejm199605163342001

Hernandez-Jover, M., Schembri, N., & Toribio, J. (2009). *Live Bird Sales Research: Qualitative study of the biosecurity practices of live bird sales and producers selling through these locations in Australia*. A Report prepared for the Department of Agriculture, Fisheries and Forestry.

Humphrey, T., & Whitehead, A. (1993). Egg age and the growth of *Salmonella enteritidis* PT4 in egg contents. *Epidemiology and infection, 111*(2), 209-220.

Humphrey, T. J., Chart, H., Baskerville, A., & Rowe, B. (1991). The influence of age on the response of SPF hens to infection with *Salmonella enteritidis* PT4. *Epidemiology and infection, 106*(1), 33-43. doi:10.1017/s0950268800056429

Humphrey, T. J., Whitehead, A., Gawler, A. H., Henley, A., & Rowe, B. (1991). Numbers of *Salmonella enteritidis* in the contents of naturally contaminated hens' eggs. *Epidemiology and infection, 106*(3), 489-496. doi:10.1017/s0950268800067546

Jia, S., McWhorter, A. R., Andrews, D. M., Underwood, G. J., & Chousalkar, K. K. (2020). Challenges in Vaccinating Layer Hens against *Salmonella* Typhimurium. *Vaccines (Basel), 8*(4). doi:10.3390/vaccines8040696

Junqueira, L., Truninger, M., Almli, V. L., Ferreira, V., Maia, R. L., & Teixeira, P. (2022). Self-reported practices by Portuguese consumers regarding eggs' safety: An analysis based on critical consumer handling points. *Food Control, 133*, 108635. doi:10.1016/j.foodcont.2021.108635

Kapperud, G., Gustavsen, S., Hellesnes, I., Hansen, A. H., Lassen, J., Hirn, J., . . . Helmuth, R. (1990). Outbreak of *Salmonella typhimurium* infection traced to contaminated chocolate and caused by a strain lacking the 60-megadalton virulence plasmid. *Journal of clinical microbiology, 28*(12), 2597-2601. doi:10.1128/jcm.28.12.2597-2601.1990

Katselos, C., & Novitski, S. (2021). *THE IMPACT OF RECENT AVIAN INFLUENZA VIRUS OUTBREAKS IN THE AUSTRALIAN POULTRY INDUSTRY.* Paper presented at the 2021 AVPA Virtual Scientific Meeting.

Kauber, K., Fowler, H., Lipton, B., Meschke, J. S., & Rabinowitz, P. (2017). *Salmonella* Knowledge, Attitudes and Practices: A Survey of Backyard Poultry Owners Residing in Seattle, Washington and the Surrounding Metropolitan Area. *Zoonoses and Public Health, 64*(1), 21-28. doi:10.1111/zph.12274

Keerthirathne, T. P., Ross, K., Fallowfield, H., & Whiley, H. (2021). Examination of Australian backyard poultry for *Salmonella*, *Campylobacter* and *Shigella* spp., and related risk factors. *Zoonoses and Public Health*. doi:10.1111/zph.12889

Kingsbury, J. M., Thom, K., Erskine, H., Olsen, L., & Soboleva, T. (2019). Prevalence and Genetic Analysis of *Salmonella enterica* from a Cross-Sectional Survey of the New Zealand Egg Production Environment. *Journal of Food Protection, 82*(12), 2201-2214. doi:10.4315/0362-028x.Jfp-19-159

Korver, D., & McMullen, L. (2017). Chapter 4 - Egg Production Systems and *Salmonella* in Canada. In S. C. Ricke & R. K. Gast (Eds.), *Producing Safe Eggs* (pp. 59-69). San Diego: Academic Press.

Lane, C., LeBaigue, S., Esan, O., Awofisyo, A., Adams, N., Fisher, I. S. T., . . . Adak, G. (2014). *Salmonella enterica* Serovar Enteritidis, England and Wales, 1945–2011. *Emerging Infectious Diseases, 20*(7), 1097. doi:10.3201/eid2007.121850

Legros, J., Jan, S., Bonnassie, S., Gautier, M., Croguennec, T., Pezennec, S., . . . Baron, F. (2021). The Role of Ovotransferrin in Egg-White Antimicrobial Activity: A Review. *Foods (Basel, Switzerland), 10*(4), 823. doi:10.3390/foods10040823

Li, S., He, Y., Mann, D. A., & Deng, X. (2021). Global spread of *Salmonella* Enteritidis via centralized sourcing and international trade of poultry breeding stocks. *Nature Communications, 12*(1), 5109. doi:10.1038/s41467-021-25319-7

Liljebjelke, K. A., Hofacre, C. L., Liu, T., White, D. G., Ayers, S., Young, S., & Maurer, J. J. (2005). Vertical and horizontal transmission of *Salmonella* within integrated broiler production system. *Foodborne Pathogens and Disease, 2*(1), 90-102. doi:10.1089/fpd.2005.2.90

Manning, J., Gole, V., & Chousalkar, K. (2015). Screening for *Salmonella* in backyard chickens. *Preventive Veterinary Medicine, 120*(2), 241-245. doi:10.1016/j.prevetmed.2015.03.019

Martelli, F., Wales, A., & Davies, R. (2017). Chapter 1 - Of Mice and Hens—Tackling *Salmonella* in Table Egg Production in the United Kingdom and Europe. In S. C. Ricke & R. K. Gast (Eds.), *Producing Safe Eggs* (pp. 3-23). San Diego: Academic Press.

McLure, A., Shadbolt, C., Desmarchelier, P. M., Kirk, M. D., & Glass, K. (2021). Source Attribution of Salmonellosis by Time and Geography in New South Wales, Australia. *Frontiers in Microbiology*.

McWhorter, A. R., & Chousalkar, K. K. (2020). *Salmonella* on Australian cage egg farms: Observations from hatching to end of lay. *Food Microbiology, 87*, 103384. doi:10.1016/j.fm.2019.103384

Messens, W., Duboccage, L., Grijspeerdt, K., Heyndrickx, M., & Herman, L. (2004). Growth of *Salmonella* serovars in hens' egg albumen as affected by storage prior to inoculation. *Food Microbiology, 21*(1), 25-32. doi:10.1016/S0740-0020(03)00045-5

Methner, U. (2013). Vaccination of poultry against *Salmonella*: what is the ideal vaccine (strain)? . Retrieved from https://www.cabi.org/Uploads/animal-science/worlds-poultry-science-association/WPSA-czech-republic-2007/8_Methner%20Ulrich.pdf

Moffatt, C. R., Musto, J., Pingault, N., Miller, M., Stafford, R., Gregory, J., . . . Kirk, M. D. (2016). *Salmonella* Typhimurium and Outbreaks of Egg-Associated Disease in Australia, 2001 to 2011. *Foodborne Pathogens and Disease, 13*(7), 379-385. doi:10.1089/fpd.2015.2110

Morse, E. V., & Duncan, M. A. (1974). Salmonellosis - an environmental health problem. *Journal of the American Veterinary Medical Association, 165*(11), 1015-1019.

MPI. (2015). Horizontal transfer and growth of *Salmonella enterica* in chicken (*Gallus gallus*) eggs in New Zealand. *MPI Technical Paper: 2015/26.* Retrieved from https://www.mpi.govt.nz/dmsdocument/9509

MPI. (2019). Egg surface survival and internalisation by New Zealand egg-associated *Salmonella* isolates as affected by storage temperature. *New Zealand Food Safety Discussion Paper No: 2019/03.* Retrieved from http://www.mpi.govt.nz/news-and-resources/publications/

MPI. (2021). New Zealand Food Safety places precautionary controls on North Island egg producer. Retrieved from https://www.mpi.govt.nz/news/media-releases/new-zealand-food-safety-places-precautionary-controls-on-north-island-egg-producer-after-detection-of-salmonella-enteritidis/

MPI. (2022). Animal Products Order: Emergency Control Scheme – Managing *Salmonella* Enteritidis in Commercial Chicken Flocks Retrieved from https://www.mpi.govt.nz/dmsdocument/47866-ECS-Managing-Salmonella-Enteritidis-in-Commercial-Chicken-Flocks

Mulqueen, K. (2021). *EMERGENCE OF SALMONELLA ENTERITIDIS IN NEW ZEALAND POULTRY 2021.* Paper presented at the 2021 AVPA Virtual Scientific Meeting.

Nicholson, C. W., Campagnolo, E. R., Boktor, S. W., & Butler, C. L. (2020). Zoonotic disease awareness survey of backyard poultry and swine owners in southcentral Pennsylvania. *Zoonoses and Public Health, 67*(3), 280-290. doi:10.1111/zph.12686

NSW Agriculture. (2003). Poultry keeping on a small scale. Retrieved from https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0005/125690/poultry-keeping-on-a-small-scale.pdf

NSW DPI. (2015). REVIEW OF EGG STAMPING IMPLEMENTATION IN NSW. Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/industry/egg_stamping_review.pdf

NSW DPI. (2020a). Biosecurity (*Salmonella* Enteritidis) Control Order 2020. Retrieved from https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0019/1149400/Biosecurity-Salmonella-Enteritidis-Control-Order-30-June-2020.pdf

NSW DPI. (2020b). How to comply with the Biosecurity (*Salmonella* Enteritidis) Control Order 2020. Retrieved from https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0006/1252482/16334-Salmonella-Control-Order-Guide_FINAL-10082020-2039.pdf

NSW DPI. (2020c). *Salmonella* Enteritidis mandatory testing: Frequently asked questions. Retrieved from https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0003/1252938/SEFAQS.pdf

NSW DPI. (2021a). Biosecurity (*Salmonella* Enteritidis) Control Order Assessment 2019-2021. Retrieved from https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0007/1345597/Biosecurity-Salmonella-Enteritidis-Control-Order-Assessment-2019-2021.pdf

NSW DPI. (2021b). *Salmonella* Enteritidis. Retrieved from https://www.dpi.nsw.gov.au/animals-and-livestock/poultry-and-birds/health-disease/salmonella-enteritidis

NSW Food Authority. (2013a). Baseline evaluation of the NSW Egg Food Safety Scheme, Microbiological survey of egg farms in NSW. Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/scienceandtechnical/egg_baseline_micro_survey.pdf

NSW Food Authority. (2013b). *Egg Food Safety Scheme, Periodic review of the risk assessment*. Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/scienceandtechnical/egg_food_safety_scheme.pdf

NSW Food Authority. (2015a). EGG CLEANING PROCEDURES, GUIDELINES TO COMPLY WITH THE EGG FOOD SAFETY SCHEME OF FOOD REGULATION 2015. Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/industry/egg_cleaning_procedures.pdf

NSW Food Authority. (2015b). NSW Food safety strategy delivers confidence. Retrieved from https://www.foodauthority.nsw.gov.au/news/departmental-media-releases/food-safety-strategy

NSW Food Authority. (2015c). NSW GOVERNMENT FOOD SAFETY STRATEGY 2015 – 2021, SAFE FOOD FROM PADDOCK TO PLATE. Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/corporate/Food_Safety_Strategy.pdf

NSW Food Authority. (2015d). REQUIREMENTS FOR EGG GRADERS. Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/industry/egg_graders.pdf

NSW Food Authority. (2015e). REQUIREMENTS FOR EGG PRODUCERS. Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/industry/egg_producers.pdf

NSW Food Authority. (2016a). FOOD SAFETY GUIDELINES FOR THE PREPARATION OF RAW EGG PRODUCTS. Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/retail/raw_egg_guidelines.pdf

NSW Food Authority. (2016b). REQUIREMENTS FOR EGG STORAGE FACILITIES. Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/industry/egg_storage.pdf

NSW Food Authority. (2016c). REQUIREMENTS FOR EGG TRANSPORT BUSINESSES. Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/industry/egg_transport.pdf

NSW Food Authority. (2017). REQUIREMENTS FOR SMALL EGG FARMS. Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/industry/egg_farms_small.pdf

NSW Food Authority. (2019a). ANNUAL FOOD TESTING REPORT 2018-2019. Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/2020-08/annual_food_testing_report_2018_2019_1.pdf

NSW Food Authority. (2019b). ANNUAL REPORT 2018–19. Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/2020-01/annual_report_2018_19.pdf

NSW Food Authority. (2020a). ANNUAL FOOD TESTING REPORT 2016 - 2017. Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/2020-08/annual_food_testing_report_2016_2017_0.pdf
NSW Food Authority. (2020b). ANNUAL FOOD TESTING REPORT 2019-2020. Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/2020-12/Annual%20Food%20testing%20report%202019%20-%20EM151220_4.20pm.pdf
NSW Food Authority. (2020c). Food Safety Schemes Manual. Retrieved from https://www.foodauthority.nsw.gov.au/industry/food-safety-schemes-manual
NSW Food Authority. (2020d). Legislation. Retrieved from https://www.foodauthority.nsw.gov.au/about-us/legislation
NSW Food Authority. (2020e). NSW consumer food safety behavioural research, SUMMARY REPORT. Retrieved from https://www.foodauthority.nsw.gov.au/sites/default/files/2020-12/NSW-consumer-food-safety-behavioural-research.pdf
NSW Food Authority. (2021a). Egg stamping. Retrieved from https://www.foodauthority.nsw.gov.au/industry/eggs/egg-stamping
NSW Food Authority. (2021b). Eggs. Retrieved from https://www.foodauthority.nsw.gov.au/industry/eggs
NSW Food Authority. (2021c). Enjoy eggs safely. Retrieved from https://www.foodauthority.nsw.gov.au/consumer/special-care-foods/eggs-enjoy-safely
NSW Food Authority. (2021d). Small egg farms. Retrieved from https://www.foodauthority.nsw.gov.au/industry/eggs/small-egg-farms
NSW Food Authority. (2022a). Food Safety Supervisors (FSS). Retrieved from https://www.foodauthority.nsw.gov.au/retail/fss-food-safety-supervisors
NSW Food Authority. (2022b). Training for food handlers. Retrieved from https://www.foodauthority.nsw.gov.au/retail/fss-food-safety-supervisors/training-for-food-handlers
NSW Government. (2018). *Antimicrobial stewardship and resistance, Policy Statement*. Retrieved from https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0005/847040/Antimicrobial-stewardship-and-resistance.PDF
O'Brien, S. J. (2013). The “Decline and Fall” of Nontyphoidal *Salmonella* in the United Kingdom. *Clinical Infectious Diseases, 56*(5), 705-710. doi:10.1093/cid/cis967
OIE. (2021). Avian Influenza. Retrieved from https://www.oie.int/en/disease/avian-influenza/
Okamura, M., Kamijima, Y., Miyamoto, T., Tani, H., Sasai, K., & Baba, E. (2001). Differences among six *Salmonella* serovars in abilities to colonize reproductive organs and to contaminate eggs in laying hens. *Avian Diseases, 45*(1), 61-69.
Pande, V. V., Gole, V. C., McWhorter, A. R., Abraham, S., & Chousalkar, K. K. (2015). Antimicrobial resistance of non-typhoidal *Salmonella* isolates from egg layer flocks and egg shells. *International Journal of Food Microbiology, 203*, 23-26. doi:10.1016/j.ijfoodmicro.2015.02.025
Pijnacker, R., Dallman, T. J., Tijsma, A. S. L., Hawkins, G., Larkin, L., Kotila, S. M., . . . Franz, E. (2019). An international outbreak of *Salmonella enterica* serotype Enteritidis linked to eggs from Poland: a microbiological and epidemiological study. *Lancet Infectious Diseases, 19*(7), 778-786. doi:10.1016/s1473-3099(19)30047-7
Pires, A. F. A., Peterson, A., Baron, J. N., Adams, R., Martínez-López, B., & Moore, D. (2019). Small-scale and backyard livestock owners needs assessment in the western United States. *PLoS One, 14*(2), e0212372. doi:10.1371/journal.pone.0212372
Quinteros, J. A., Wilson, T. B., Anwar, A. M., Scott, T., Shil, P., Noormohammadi, A. H., & Scott, P. C. (2021). *EVALUATION OF VACCINATION PROGRAM OPTIONS AGAINST SALMONELLA ENTERITIDIS 7A IN LAYING HENS.* Paper presented at the 2021 AVPA Virtual Scientific Meeting.
Raspoet, R., Gantois, I., Devloo, R., Pasmans, F., Haesebrouck, F., Ducatelle, R., & Van Immerseel, F. (2011). Internal contamination of eggs by *Salmonella* Enteritidis. In F. Van Immerseel, Y. Nys, & M. Bain (Eds.), *Improving the Safety and Quality of Eggs and Egg Products* (pp. 46-61): Woodhead Publishing.
Safe Food Production Queensland. (2015). 2015 *Salmonella* survey of the Queensland egg production environment. Retrieved from http://esvc000111.wic055u.server-web.com/images/PDF/2015%20Microbiological%20survey%20of%20Queensland%20egg%20farms.pdf
Scott, A. B., Singh, M., Groves, P., Hernandez-Jover, M., Barnes, B., Glass, K., . . . Toribio, J. A. (2018). Biosecurity practices on Australian commercial layer and meat chicken farms: Performance and perceptions of farmers. *PLoS One, 13*(4), e0195582. doi:10.1371/journal.pone.0195582
Scott, P. C., Wilson, T., Quinteros, J. A., Anwar, A., Scott, T., Shill, P., & Noormohammadi, A. H. (2020). Evaluation of vaccination program options against *Salmonella* Enteritidis 7A in laying hens in Australia. *Final Project Report, OCTOBER 2020.* Retrieved from https://www.australianeggs.org.au/assets/research/documents/Evaluation-of-vaccination-program-options-against-Salmonella-Enteritidis-7A-in-laying-hens-in-Australia-Final-Report.pdf

Scott, P. C., Wilson, T., Quinteros, J. A., Anwar, A., Scott, T., Shill, P., & Noormohammadi, A. H. (2021). Evaluation of the duration of immunity induced by a vaccination program against *Salmonella* Enteritidis 7A in laying hens in Australia (Stage 2). *Final Project Report, JULY 2021.* Retrieved from https://www.australianeggs.org.au/assets/research/documents/For-website-FINAL-REPORT-Evaluation-of-the-duration-of-immunity-induced-by-a-vaccination-program-against-SE-7A.-Stage-2.pdf

Sharma, P., Caraguel, C., Sexton, M., McWhorter, A., Underwood, G., Holden, K., & Chousalkar, K. (2018). Shedding of *Salmonella* Typhimurium in vaccinated and unvaccinated hens during early lay in field conditions: a randomised controlled trial. *BMC microbiology, 18*(1), 78. doi:10.1186/s12866-018-1201-0

Sodagari, H. R., Habib, I., Whiddon, S., Wang, P., Mohammed, A. B., Robertson, I., & Goodchild, S. (2020). Occurrence and Characterization of *Salmonella* Isolated from Table Egg Layer Farming Environments in Western Australia and Insights into Biosecurity and Egg Handling Practices. *Pathogens, 9*(1). doi:10.3390/pathogens9010056

Sodagari, H. R., Mohammed, A. B., Wang, P., O'Dea, M., Abraham, S., Robertson, I., & Habib, I. (2019). Non-typhoidal *Salmonella* contamination in egg shells and contents from retail in Western Australia: Serovar diversity, multilocus sequence types, and phenotypic and genomic characterizations of antimicrobial resistance. *International Journal of Food Microbiology, 308*, 108305. doi:10.1016/j.ijfoodmicro.2019.108305

Sodagari, H. R., Wang, P., Robertson, I., Abraham, S., Sahibzada, S., & Habib, I. (2021). Antimicrobial resistance and genomic characterisation of *Escherichia coli* isolated from caged and non-caged retail table eggs in Western Australia. *International Journal of Food Microbiology, 340*, 109054. doi:10.1016/j.ijfoodmicro.2021.109054

Souvestre, M., Delpont, M., Guinat, C., Dumat, C., Guichard, L., Manis, L., . . . Le Loc'h, G. (2021). Backyard poultry flocks in France: A diversity of owners and biosecurity practices. *Preventive Veterinary Medicine, 197*, 105511. doi:10.1016/j.prevetmed.2021.105511

Thomas, C., Daughtry, B., Padula, D., Jordan, D., Arzey, G., Davey, K., . . . Pointon, A. (2006). An Egg: *Salmonella* Quantitative Risk Assessment Model for the Australian Egg Industry.

Trampel, D. W., Holder, T. G., & Gast, R. K. (2014). Integrated farm management to prevent *Salmonella* Enteritidis contamination of eggs. *Journal of Applied Poultry Research, 23*(2), 353-365. doi:10.3382/japr.2014-00944

UK Health Security Agency. (2021a). Non-typhoidal *Salmonella* data 2008 to 2017. Retrieved from https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1026150/salmonella-annual-report-2017.pdf

UK Health Security Agency. (2021b). Non-typhoidal *Salmonella* data 2009 to 2018. Retrieved from https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1026149/salmonella-annual-report-2018.pdf

UK Health Security Agency. (2021c). Non-typhoidal *Salmonella* data 2010 to 2019. Retrieved from https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1026208/salmonella-annual-report-2019.pdf

USDA. (2014a). Layers 2013, Part II: Control and Prevention of *Salmonella* Enteritidis on Table-Egg Farms in the United States, 2013. Retrieved from https://www.aphis.usda.gov/animal_health/nahms/poultry/downloads/layers2013/Layers2013_dr_PartII.pdf

USDA. (2014b). *Salmonella* Enteritidis on Table Egg Farms in the United States. Retrieved from https://www.aphis.usda.gov/animal_health/nahms/poultry/downloads/layers2013/Layers2013_is_SE.pdf

USDA. (2019). Food Product Dating. Retrieved from https://www.fsis.usda.gov/food-safety/safe-food-handling-and-preparation/food-safety-basics/food-product-dating

USDA. (2021). How We Store Our Eggs—and Why. Retrieved from https://tellus.ars.usda.gov/stories/articles/how-we-store-our-eggs-and-why/

van de Reep, L., Nielen, M., Verstappen, K. M. H. W., Broens, E. M., van den Broek, J., & Velkers, F. C. (2018). Response to a *Salmonella* Enteritidis challenge in old laying hens with different vaccination histories. *Poultry Science, 97*(8), 2733-2739. doi:10.3382/ps/pey134

Van Immerseel, F., De Buck, J., Pasmans, F., Bohez, L., Boyen, F., Haesebrouck, F., & Ducatelle, R. (2004). Intermittent long-term shedding and induction of carrier birds after infection of chickens early posthatch with a low or high dose of *Salmonella* Enteritidis. *Poultry Science, 83*(11), 1911-1916. doi:10.1093/ps/83.11.1911

Veltman, T., Jordan, D., McDevitt, C. A., Bell, J., Howden, B. P., Valcanis, M., . . . Trott, D. J. (2021). Absence of high priority critically important antimicrobial resistance in *Salmonella* sp. isolated from Australian commercial egg layer environments. *International Journal of Food Microbiology, 340*, 109042. doi:10.1016/j.ijfoodmicro.2021.109042

Wales, A., Breslin, M., & Davies, R. (2006). Assessment of cleaning and disinfection in *Salmonella*-contaminated poultry layer houses using qualitative and semi-quantitative culture techniques. *Veterinary Microbiology, 116*(4), 283-293. doi:10.1016/j.vetmic.2006.04.026

Waltman, D. (2017). Chapter 9 - Developments in Detection Strategies for *Salmonella* Enteritidis in Layer Hen Flocks. In S. C. Ricke & R. K. Gast (Eds.), *Producing Safe Eggs* (pp. 161-185). San Diego: Academic Press.

Whiley, H., Clarke, B., & Ross, K. (2017). Knowledge and Attitudes towards Handling Eggs in the Home: An Unexplored Food Safety Issue? *International Journal of Environmental Research and Public Health, 14*(1), 48.

Whiting, R. C., Hogue, A., Schlosser, W. D., Ebel, E. D., Morales, R. A., Baker, A., & McDowell, R. M. (2000). A Quantitative Process Model for *Salmonella* Enteritidis in Shell Eggs. *Journal of Food Science, 65*(5), 864-869. doi:10.1111/j.1365-2621.2000.tb13601.x

Zhang, X., Payne, M., Wang, Q., Sintchenko, V., & Lan, R. (2020). Highly Sensitive and Specific Detection and Serotyping of Five Prevalent *Salmonella* Serovars by Multiple Cross-Displacement Amplification. *Journal of Molecular Diagnostics, 22*(5), 708-719. doi:10.1016/j.jmoldx.2020.02.006