New Zealand Food Safety
Haumaru Kai Aotearoa

Risk Profile update: *Salmonella* (non-typhoidal) in and on eggs

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Scientific Interpretative Summary

This Scientific Interpretive Summary (SIS) is prepared by New Zealand Food Safety (NZFS) risk assessors to provide context to the following report for MPI risk managers and external readers.

NZFSSRC406922-M09/FW22037: Risk Profile update: *Salmonella* (non-typhoidal) in and on eggs

The detection of a strain of *Salmonella* Enteritidis through the routine National Microbiological Database (NMD) monitoring programme in March 2021 was the first reported incidence in New Zealand commercial poultry flocks. The strain was linked by whole genome sequencing (WGS) to human cases, including an outbreak dating back to Dec 2019, with a seemingly higher rate of hospitalisation than other *Salmonella* strains. The strain detected in poultry and causing illness in people was determined to be a potentially transovarian strain phage type (PT) 8, which could have particularly concerning consequences for egg farmers.

MPI's subsequent response was two-fold: to minimise risk of illness to consumers of poultry products, and to protect the reputation of New Zealand's poultry industry. Early investigation showed that implementation of poultry industry guidelines, particularly regarding biosecurity, were inconsistent. There was complacency in some quarters towards the risk of *Salmonella* where prevention relied on populating sheds with *Salmonella*-free birds, without sufficient risk management measures in place throughout the supply chain. An emergency control scheme was put in place, which ultimately lead to an amendment to the Animal Products Notice: Production, Supply and Processing, requiring poultry chain operators to include risk management steps specifically for *Salmonella* Enteritidis as part of their Risk Management Programme (RMP).

The original Risk Profile for *Salmonella* (non-typhoidal) in and on eggs in a New Zealand context was published in 2004, with updates in 2011 and 2016. As these were published prior to *Salmonella* Enteritidis detection in commercial poultry, the contribution to human illness attributed to New Zealand-grown poultry from *Salmonella* Enteritidis was not considered. The Risk Profile on *Salmonella* (non-typhoidal) in and on eggs was updated to consider any potential change in the risk of salmonellosis from eggs produced in New Zealand. The risk management questions it set out to answer were to help understand whether the risk to public health from consumption of eggs had changed since the previous risk profile, and how the implementation of new risk management regulations may affect the risk of human salmonellosis.

The risk associated with non-Enteritidis *Salmonella* serotypes remains low in New Zealand. This conclusion is based on a low prevalence of non-Enteritidis serotypes in New Zealand layer flocks in a 2016 survey, the static incidence of salmonellosis, and few outbreaks involving non-Enteritidis serotypes where eggs were suspected. Experimental evidence showed *Salmonella* numbers reduce with time on clean eggshells at New Zealand-relevant storage temperatures and trans-shell transmission into egg contents has not been shown.

NZFS also considers that while the risk associated with *Salmonella* Enteritidis is no longer negligible, it remains low in New Zealand. The emergence of a *Salmonella* Enteritidis strain in poultry has not yet had a material impact on overall salmonellosis case numbers in New Zealand. Information to date suggests that the regulatory measures implemented by NZFS (Emergency Control Scheme, and 2022 additions to the Animal Products Act 1999) requiring breeder, egg layer and meat chicken producers to implement more stringent risk management procedures have been effective at controlling the incidence of *Salmonella* Enteritidis on farms and hence mitigating the risk of *Salmonella* in eggs to consumers.

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RISK PROFILE UPDATE: *SALMONELLA* (NON-TYPHOIDAL) IN AND ON EGGS

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ABBREVIATIONS

2002CNS	The 2002 National Childrens' Nutrition Survey
2009ANS	The 2009 Adult Nutrition Survey
CFU	colony forming units
CI	confidence interval
CIDT	culture-independent diagnostic tests
CLSI	Clinical and Laboratory Standards Institute
DALY	disability adjusted life years
DHB	District Health Board
DT	definitive phage type
D-value	The time of exposure at a given temperature/treatment that results in a 90% (a decimal or 1 $\log_{10}$ cycle) reduction in the number of viable organisms
EAP	Export Approved Premises
ECS	Emergency Control Scheme
EFSA	European Food Safety Authority
EOL	End-of-lay
EPF	Egg Producers Federation of New Zealand Inc
ERL	Enteric Reference Laboratory (at ESR)
ESR	Institute of Environmental Science and Research Limited
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization of the United Nations
FSANZ	Food Standards Australia New Zealand
FSIS	Food Safety and Inspection Service (USDA)
HACCP	Hazard Analysis and Critical Control Point
MLST	Multilocus sequence type
MLVA	Multiple-locus variable-number tandem repeat analysis
MPI	Ministry for Primary Industries
MPN	Most probable number
MS	Member states
NMD	National Microbiological Database

NSEMAP	National *Salmonella* Enteritidis Monitoring and Accreditation Program
NSW	New South Wales (Australia)
NZFS	New Zealand Food Safety
OMAR	Overseas Market Access Requirements
OR	Odds ratio
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PFGE	pulsed field gel electrophoresis
PIANZ	Poultry Industry Association of New Zealand
PT	provisional phage type
RDNC	reaction does not conform
RH	relative humidity
RMP	Risk Management Programme
RMQ	Risk Management Question
RTE	ready-to-eat
SE_2019_C_01	*S.* Enteritidis outbreak strain designation
SNP	single nucleotide polymorphism
ST	sequence type
TP	true prevalence
UK	United Kingdom
US	United States of America
US CDC	United States Centers for Disease Control and Prevention
USDA	United States Department of Agriculture
USFDA	United States Food and Drug Administration
VBNC	viable but non-culturable
YMT	Yolk Mean Time
WGS	whole genome sequencing
WHO	World Health Organization

SUMMARY

The purpose of a Risk Profile is to provide information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. Risk Profiles include elements of a qualitative risk assessment, as well as providing information relevant to risk management. This Risk Profile concerns non-typhoidal *Salmonella enterica* subspecies *enterica* (hereafter referred to as *Salmonella*) in and on chicken eggs. This is an update of a Risk Profile published in 2016 (Rivas et al. 2016). The key finding from that report was that based on the available data, the public health risk from *Salmonella* in or on eggs consumed in New Zealand had not changed since an earlier 2011 Risk Profile. Whole, fresh eggs sold in New Zealand could be contaminated with *Salmonella*, but this was contributing to only a minor (but undefined) proportion of human illness.

The majority of commercial whole egg and egg product available in New Zealand is from domestically farmed chickens, while a very small number of enterprises produce eggs from ducks and quail. In 2022, there were approximately 1.2 billion chicken eggs produced from a per-month average of 3.6 million layer hens, which was similar to 2015 numbers. However, production was higher in intervening years, with up to 4.2 million birds in lay in 2020. A new Code of Welfare requiring phased prohibition of conventional cages by the end of 2022, together with a commitment by some supermarkets to only sell cage-free eggs by the end of 2025, has impacted egg production recently. Much smaller quantities of eggs or egg product are exported and imported each year; there has been a 44% reduction in egg and egg product exports since 2015 (1,100 tonnes in 2022), while imported egg products have almost tripled (983 tonnes in 2022). Fresh eggs are not imported. The majority of eggs are sold in New Zealand as fresh, whole eggs but liquid and dried egg products are also available.

Poultry can be exposed to *Salmonella* from a range of sources, and colonisation primarily occurs via the faecal-oral route. *Salmonella* from colonised chickens can contaminate eggs, both externally (all serotypes) and in egg contents. Contaminated eggs from breeder chickens pose a risk of colonisation of unhatched and newly hatched layer chicks. Contaminated eggs from layer chickens pose a risk to consumers. Contamination of egg contents can occur when *Salmonella* (most serotypes) penetrates through the eggshell into the contents, or through transovarian transmission when *Salmonella* (primarily, the invasive serotype *S.* Enteritidis) present in the reproductive tract migrates into egg contents before full egg formation. *S*. Enteritidis is the dominant serotype in European and North American layer flocks and is the cause of the majority of human salmonellosis attributed to eggs in these regions. Certain phage types of *S*. Enteritidis such as DT8 have been reported to be capable of transovarian contamination of eggs, but the genetic determinants for what makes a strain capable of this are not well understood. The first detection of *S*. Enteritidis ST11, DT8 in New Zealand poultry was from a processed poultry carcass during 2021, followed by detection in hatcheries and poultry sheds (from both layer and broiler flocks) from which the birds associated with the carcass meat detection were originally sourced.

Salmonella will not grow on the eggshells of clean eggs and will decrease in numbers over time. The rate of decrease depends on the serotype present and the storage temperature. Survival of *Salmonella* on eggs has been reported to be better under refrigeration than at

higher temperatures. A study that inoculated egg surfaces with New Zealand egg-associated non-Enteritidis *Salmonella* isolates found that *Salmonella* viability declined more rapidly on eggshells at 22˚C compared with 15˚C. However, faecal contamination on eggshells significantly increased *Salmonella* survival, emphasising the need to ensure that eggs are sold clean. Contamination of egg contents was not observed in this study, which supports that internalisation into unwashed, intact eggs, and survival for the duration of egg storage is a rare event. Whole eggs inhibit bacterial contamination of the contents through physical barriers (cuticle, shell, membranes), and antimicrobial components inhibit growth from occurring in the albumen. However, *S*. Enteritidis has been reported to survive better in albumen than other serotypes. *Salmonella* may reach the egg yolk by migrating through the albumen and across the vitelline membrane surrounding the yolk. The vitelline membrane breaks down over time. The egg yolk supports *Salmonella* growth in a temperature-dependent manner.

The yearly incidence of salmonellosis in New Zealand was relatively static for the period covered by this Risk Profile (from 2015 to 2022), and slightly lower compared with the period (2005 to 2014) covered in the 2016 Risk Profile. There were fewer notifications during 2020 and 2021 than earlier years which could be attributed to the impact of the public health response to the COVID-19 pandemic. New Zealand rates of salmonellosis are similar to those of European Union (EU) and United States (US), and lower than in Australia. Just a single death associated with salmonellosis occurred in New Zealand during this time (in 2017). Antimicrobial resistance remains relatively low among non-typhoidal *Salmonella* isolated from human, animal and environmental samples in New Zealand. *S*. Typhimurium was the most frequently isolated serotype from human salmonellosis cases in New Zealand, followed by *S.* Enteritidis (38.2% and 12.0%, respectively, for the period 2015 to 2022).

From 2015 to 2021, there were six salmonellosis outbreaks where eggs were suspected or confirmed as the vehicle of infection, including 79 confirmed and 24 probable cases. Of these, most cases were part of a single outbreak where there was strong evidence for transmission associated with eggs. This was caused by the *S*. Enteritidis DT8, ST11 strain (designated SE_2019_C_01) also identified in poultry flocks. Additional cases were reported in 2022 and 2023, so that from May 2019 to February 2023, this outbreak included 128 confirmed outbreak cases and six additional epidemiologically linked cases (134 total cases). Of the 134 cases, 37% of cases were hospitalised, which was a higher percentage than for all salmonellosis cases (27%) or total *S*. Enteritidis cases (28%) over a similar reporting period.

In response to the *S*. Enteritidis DT8, ST11 outbreak associated with poultry, a new regulatory framework was introduced for all sectors (breeders, hatcheries, rearer, broiler and layer farms) within the poultry industry. Requirements include a Risk Management Programme for all sectors of the industry; microbiological testing of the poultry environment for *S*. Enteritidis; procedures for the tracing and management of *S*. Enteritidis from the poultry supply chain where detected; and changes to Overseas Market Access Requirements.

This Risk Profile seeks to answer the following specific Risk Management Questions (RMQs), with a focus on information that has become available since the 2016 update was produced:

- **RMQ1:** Considering the detection of *Salmonella* Enteritidis in chicken hatcheries/day-one chicken suppliers in 2021, how has the public health risk from *Salmonella* in or on eggs changed since the 2016 Risk Profile update?

Detection of the *S*. Enteritidis DT8, ST11 strain SE_2019_C_01 in layer flocks has the potential to increase the risk to the New Zealand layer industry and to consumers of eggs. The potential for transovarian transmission of *S*. Enteritidis to eggs via the breeder flocks at the apex of the supply chain could result in widespread dissemination through the layer poultry supply chain. Colonisation of layer flocks poses a greater risk for consumers because egg contents are more likely to be contaminated via transovarian transmission by *S.* Enteritidis than by other *Salmonella* serotypes. This was the only foodborne salmonellosis outbreak over the period assessed where there was strong evidence for eggs as a vehicle, although poultry meat was also considered a potential source.. There is some evidence that this strain poses a greater risk to human health than other *Salmonella* serotypes because of a higher hospitalisation rate. There is also a risk to international trade in hatching and table eggs.

The residual level of risk will be determined by the efficacy of the new control measures implemented to detect flock colonisation, eliminate colonised flocks, and control dissemination of *S*. Enteritidis. Although this strain has the potential to increase the risk to consumers of eggs, the absence of reported cases of infection with the outbreak strain since February 2023 suggests that risk management procedures have been effective at controlling the risk.

The risk associated with non-Enteritidis *Salmonella* serotypes in and on eggs does not appear to have changed since the 2016 Risk Profile. This conclusion is based on a low prevalence of non-Enteritidis serotypes in New Zealand layer flocks in a 2016 survey, the static incidence of salmonellosis, and few outbreaks involving non-Enteritidis serotypes where eggs were suspected. Detection of non-Enteritidis serotypes from egg contact surfaces in New Zealand packhouses show that eggs can potentially be contaminated by these serotypes. However, experiments showed that they die over time on clean eggshells at New Zealand-relevant storage temperatures and trans-shell transmission into egg contents was not detected.

Important knowledge gaps include:

- Current prevalence data for non-Enteritidis *Salmonella* in layer poultry breeders, hatcheries, and layer flocks; and how the increasing proportions of hens housed in cage-free systems are influencing prevalence.
- Whether the *S*. Enteritidis SE_2019_C_01 strain is indeed capable of transovarian transmission, and the behaviour of this strain in and on eggs at New Zealand-relevant storage temperatures.
- Whether *S*. Enteritidis SE_2019_C_01 is disseminated in the wider environment and if there are unknown reservoirs (such as backyard poultry).
- The route by which *S*. Enteritidis SE_2019_C_01 was introduced into the New Zealand poultry industry.
- Lack of recent national nutrition surveys to assess poultry consumption trends and apportion consumption data for at risk demographics.

- **RMQ2:** What interventions are available to manage the risk from *Salmonella* in and on eggs and what is known about their effectiveness?

The most effective overall strategy to control *Salmonella* in and on eggs is by applying multiple interventions throughout the egg production chain to control colonisation of layer chickens and prevent contamination of the farm environment. Environmental management includes controlling the food and water supply, biosecurity and pest management, and ensuring effective cleaning regimes are in place. Vaccination is widely practiced on New Zealand layer farms, and can reduce, but not prevent, flock colonisation, shedding, and contamination of eggs. Adding prebiotics, probiotics, bacteriophages, organic acids or phytochemicals to feed for hens has been shown to provide some protection against *Salmonella*.

Post-harvest control measures may include egg washing/sanitising or UV treatment of eggs, which can reduce *Salmonella* numbers on egg surfaces. Pasteurisation or fully cooking eggs inactivates *Salmonella* in egg contents. Other effective hazard mitigation behaviours for consumers include discarding eggs that are dirty, cracked or past their best before date, and washing hands and surfaces following contact with raw eggs. Refrigerating eggs post-lay will control the growth of any *Salmonella* that might be present in the egg contents.

- **RMQ3:** What information is available to advise industry regarding shelf life and storage conditions for eggs in relation to the risk from *Salmonella*?

The current shelf life for New Zealand eggs is 35 days (shown as a best before date) regardless of storage temperature. *Salmonella* present on clean eggshells will not grow, and will die faster at warmer storage temperatures (for example, room temperature compared with refrigeration). However, warmer temperatures promote faster breakdown of the vitelline membrane and more rapid growth of any *Salmonella* present in egg yolk, whereas *Salmonella* will not grow at refrigeration temperatures. New Zealand shelf life considerations were guided by the very low likelihood that *Salmonella* would be present in egg contents, but the risk for contamination of egg contents is higher for *S*. Enteritidis because it is potentially transovarian. Current data suggest that the risk management interventions are effectively mitigating *S*. Enteritidis in New Zealand layer flocks. However, a reconsideration of shelf life guidelines would be important if the strain were to re-emerge and become endemic in New Zealand layer flocks. A knowledge of whether *S*. Enteritidis SE_2019_C_01 is capable of transovarian transmission, and the behaviour of this strain in and on eggs at New Zealand-relevant storage temperatures, including under refrigeration, would inform modelling for shelf life considerations.

- **RMQ4:** What is the best way to gather information on the prevalence of *Salmonella* in New Zealand eggs?

The best approach to gather information on the prevalence of *Salmonella* on New Zealand eggs is by environmental sampling of dust and faeces in layer sheds. Testing egg contact surfaces at packhouses can also indicate that contamination of egg surfaces is occurring. The newly implemented testing programme for *S*. Enteritidis in New Zealand breeder, layer and broiler flocks and hatcheries has been designed to maximise the likelihood of *S*. Enteritidis detection if it is present in flocks. The testing programme appears as rigorous as that

conducted in the European Union with respect to sampling frequency, timing, and sensitivity of sample types. However, the testing does not cover the risk of egg contamination from other *Salmonella* serotypes. Testing regulatory framework samples for total *Salmonella* prevalence, and targeting other serotypes of higher concern such as *S*. Typhimurium in addition to *S*. Enteritidis, would provide valuable information on the risk of all *Salmonella* serotypes to New Zealand eggs.

1 INTRODUCTION

The purpose of a Risk Profile is to provide information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. Risk Profiles are a preliminary risk management activity, and part of the Risk Management Framework (RMF)[1] approach taken by New Zealand Food Safety (NZFS), part of the Ministry for Primary Industries (MPI). The Framework consists of a four-step process:

- Preliminary risk management activities;
- Identification and selection of risk management options;
- Implementation of control measures; and
- Monitoring and review.

This Risk Profile considers non-typhoidal *Salmonella* in layer hens and eggs. This is an update of a 2011 Risk Profile (Lake et al. 2011) and an update to that document published in 2016 (Rivas et al. 2016). As such, this is not a stand-alone document.

The hazard considered in this Risk Profile is the group of non-typhoidal serotypes of the bacteria *Salmonella enterica* subspecies *enterica*. *Salmonella* serotypes that are adapted to animals other than poultry, such as *S.* Choleraesuis (pig-adapted), are excluded. Similarly, typhoidal *Salmonella* serotypes *S. enterica* subspecies *enterica* (*S.*) Typhi, *S*. Paratyphi A, B, and C, and *S*. Sendai are not considered by this Risk Profile as they are restricted to human hosts (Feng et al. 2019, Gal-Mor 2019). The exception is Paratyphi B variant Java[2], which is considered because it is a dominant poultry serotype overseas (van Pelt et al. 2003, Castellanos et al. 2020).

The food considered in this Risk Profile is chicken eggs that are commercially produced in New Zealand from laying hens of the species *Gallus gallus*. Eggs harvested from backyard poultry for personal consumption are excluded, as are eggs from other types of poultry such as quail, geese, ducks, emus and ostriches due to the small size of the market share.

This Risk Profile seeks to answer the following specific Risk Management Questions (RMQs), with a focus on information that has become available since the 2016 Risk Profile (Rivas et al. 2016) was produced:

- **RMQ1:** Considering the detection of *Salmonella* Enteritidis in chicken hatcheries/day-one chicken suppliers in 2021, how has the public health risk from *Salmonella* in or on eggs changed since the 2016 Risk Profile update?
- **RMQ2:** What interventions are available to manage the risk from *Salmonella* in and on eggs and what is known about their effectiveness?
- **RMQ3:** What information is available to advise industry regarding shelf life and storage conditions for eggs in relation to the risk from *Salmonella*?
- **RMQ4:** What is the best way to gather information on the prevalence of *Salmonella* in New Zealand eggs?

[1] https://www.mpi.govt.nz/dmsdocument/22000/send, accessed 27 October 2022
[2] S. Java is also known more recently as *S*. enterica subsp. enterica serovar Paratyphi B var. d-Tartrate$^+$

Additionally, the report identifies data gaps and areas for potential research to inform MPI's ongoing *S*. Enteritidis risk management programme.

2 HAZARD AND FOOD

2.1 THE PATHOGEN: NON-TYPHOIDAL *SALMONELLA*

Key findings

- All *Salmonella* serotypes are considered potentially pathogenic to humans. Pathogenicity varies between and within serotypes.
- The primary sources of *Salmonella* are the gastrointestinal tracts of humans and animals, via excretion into the environment.
- Red and white meats, meat products, unpasteurised milk, raw milk cheeses and eggs are the foods most often implicated as causes of human salmonellosis, although a wide variety of other foods have also been associated with outbreaks.
- *Salmonella enterica* subspecies *enterica* serotype Enteritidis (*S*. Enteritidis) continues to be recognised as the dominant serotype in layer flocks in European and North American countries, and is the cause of the majority of human infections attributed to eggs in these regions. *S*. Enteritidis can colonise the reproductive organs of hens and contaminate eggs prior to shell formation. *S*. Enteritidis was detected in New Zealand poultry for the first time in 2021.

2.1.1 *Salmonella* species (spp.)

This group of bacteria is comprised of two species: *Salmonella enterica*, which is divided into six subspecies (*enterica, salamae, arizonae, diarizonae, houtenae* and *indica*), and *Salmonella bongori* (Grimont and Weill 2007). Most pathogenic isolates from humans and other mammals belong to *S. enterica* subspecies *enterica*. Other *S. enterica* subspecies and *S. bongori* are more common in cold blooded animals and the environment and are of lower pathogenicity to humans and livestock (Brenner et al. 2000, Lamas et al. 2018).

Salmonella are primarily divided into types using serological identification of somatic (O), flagella (H), and capsular (K) antigens, which are named according to the Kaufmann-White scheme (last updated in 2007) (Grimont and Weill 2007). There are more than 2,500 different *Salmonella* serotypes (also called serovars), and of these over 1,500 have been identified in the *S*. enterica subspecies enterica group. For designating the serotype, the subspecies does not need to be indicated, but it has been recommended that the abbreviation (*S*.) of the genus name (*Salmonella*) should not stand alone without being followed by a specific epithet (*S. enterica*) (Grimont and Weill 2007). However, for practical purposes, the approach taken by this report, and commonly used in literature, involves abbreviating *S. enterica* subspecies *enterica* serotypes to a shortened form after the first citation (Brenner et al. 2000). The serotype is capitalised and non-italicised; for example, *Salmonella enterica* subsp. *enterica* serotype Enteritidis becomes *Salmonella* Enteritidis (or *S.* Enteritidis) (Brenner et al. 2000).

Previous Risk Profiles have referred to "*Salmonella* spp.". Technically, spp. refers to multiple *Salmonella* species, and for the large part, we are only referring to the single subspecies (*S. enterica* subspecies *enterica*), which is defined in the previous paragraph. As such, in this document "*Salmonella*" is used throughout, rather than "*Salmonella* spp.".

Salmonella can be further subtyped by measuring susceptibility to a panel of bacteriophages. These types are denoted as provisional phage type (PT) or definitive phage type (DT) numbers; the term DT is used in this document. Phage typing does not provide information on genotypic relationships and has been phased out in favour of more informative molecular analyses. The production of phages for *Salmonella* phage typing has now been discontinued internationally.

Since November 2019, whole genome sequence (WGS)-based typing methods have replaced phage typing for typing of *S.* Enteritidis and *S.* Typhimurium isolates in New Zealand. These isolates are now reported as the serotype and an Achtman 7-gene multilocus sequence type (MLST; or more simply, sequence type; ST) is now reported (Achtman et al. 2012). Unlike phage typing, the ST enables isolates to be clustered into evolutionary groupings. There is no correlation between phage type and sequence type because closely related *Salmonella* strains might have different phage types, and not all strains of the same phage type are closely related (Pang et al. 2012, Mohammed and Cormican 2015, Kingsbury and Soboleva 2019b). WGS data can be used to investigate the relatedness of strains of the same serotype and ST. This WGS-fine typing involves Single Nucleotide Polymorphism (SNP) analysis. This method is now used in New Zealand and internationally for salmonellosis outbreak or cluster investigations. Further information on *Salmonella* typing, is included in Appendix A.2.

2.1.2 Sources of *Salmonella*

The primary sources of *Salmonella* are the gastrointestinal tracts of humans and animals, meaning that the pathogen is widespread in the environment (Bell and Kyriakides 2002).

Since the 2016 Risk Profile (Rivas et al. 2016), the main change to the primary sources and transmission of *Salmonella* within New Zealand with relevance to poultry is the detection of *S.* Enteritidis DT8, ST11 in the New Zealand layer and broiler flocks during 2021.

Humans: Person-to-person transmission of *Salmonella* is well recognised, and secondary transmission of *Salmonella* in outbreaks has been demonstrated (Loewenstein 1975). Carriage in faeces in convalescent cases can be quite substantial with numbers approximating 10^6-10^7 *Salmonella*/g faeces persisting up to 10 days after initial diagnosis. Reduction in numbers with time is variable; most people will have counts of less than 100 *Salmonella*/g faeces after 35 to 40 days, but a count of 6 x 10^3/g has been recorded in one patient 48 days post-illness (Pether and Scott 1982). In New Zealand, other gastrointestinal diseases such as cryptosporidiosis, giardiasis and shigellosis are more strongly associated with person-to-person transmission than salmonellosis, but person-to-person risk factors are commonly d in outbreak reports (Adlam et al. 2010). Asymptomatic carriage may also occur, and asymptomatic food handlers have been responsible for an outbreak of hospital-acquired infection (Dryden 1994) as well as an outbreak in a catering establishment (Stein-Zamir et al. 2009). An Australian study found a prevalence of 0.4% amongst 1,091 asymptomatic people (Hellard et al. 2000).

Animals: *Salmonella* can be found in mammals, fish, reptiles, amphibians, insects and birds. Most *Salmonella* colonisations in animals produce no clinical signs. Some serotypes are largely confined to particular animal reservoirs causing both systemic and enteric disease, for

example *S*. Choleraesuis is host-restricted to pigs (Allison et al. 1969, Uzzau et al. 2000, Jajere 2019). Other serotypes such as *S.* Typhimurium are considered to have a broad host range because they are frequently detected in the intestinal contents of a wide range of animal species (Paulin et al. 2002). However, variants within the *S*. Typhimurium serotype can differ significantly in their host range and their degree of host adaptation (Rabsch et al. 2002). Animal feed ingredients may be contaminated with *Salmonella*, serving as a source for animal colonisation. Sick animals have been the source of sporadic human salmonellosis cases (Adlam et al. 2010).

Food: *Salmonella* are an important cause of foodborne illness worldwide. Foods and ingredients become contaminated through contact with faecal material, either directly or via environmental sources (for example, water and soil). The foods most commonly implicated as sources of human infection are red and white meats, meat products, unpasteurised milk, cheese and eggs (Jay et al. 2003, Chanamé Pinedo et al. 2022). Globally, *S.* Typhimurium is widespread in foods produced from animals, while *S.* Enteritidis tends to be associated with poultry products and *S.* Anatum with beef products (Ferrari et al. 2019).

In Australia, *S*. Typhimurium is the most commonly identified serotype in foodborne salmonellosis outbreaks; these outbreaks are most frequently associated with the consumption of raw or undercooked eggs, although poultry meat is also often implicated (OzFoodNet Working Group 2022). *S*. Enteritidis can colonise the reproductive organs of hens and contaminate eggs prior to shell formation (transovarian transmission, discussed in more detail in Section 2.3). In Europe, *S*. Enteritidis is the most common serotype in layer flocks (and the second most common in broiler flocks), and continues to be the most common serotype in outbreaks, followed by *S*. Typhimurium (European Food Safety Authority and European Centre for Disease Prevention and Control 2022). The foods implicated most often in salmonellosis outbreaks occurring in Europe are eggs and egg products, as well as mixed foods (meals composed of various ingredients), and to a lesser extent poultry meat (De Knegt et al. 2015, European Food Safety Authority and European Centre for Disease Prevention and Control 2022). In contrast, New Zealand has a very low reported incidence of egg-associated salmonellosis, and *S*. Enteritidis had not been detected in New Zealand poultry until 2021 (see section 3.3) (Ministry for Primary Industries 2015, Kingsbury et al. 2019a).

A wide variety of other foods have been associated with salmonellosis outbreaks. These include seafood (shellfish, salmon), nuts and nut products (desiccated coconut, peanut butter), cereal and cereal products (barley, cereal powder), spices (white and black pepper, paprika), oilseeds and oilseed products (cottonseed, soybean sauce, sesame seeds), vegetables (watercress, tomatoes, lettuce, potato and other salads, bean and alfalfa sprouts), fruit and fruit products (watermelon, melon, cider) and other miscellaneous products (chocolate, cocoa powder, dried yeast, candy). Due to the ability of *Salmonella* to survive in foods with low water activity (Finn et al. 2013), outbreaks have occurred in New Zealand and internationally from *Salmonella*-contaminated flour and tahini (a product made from crushed sesame seeds) (Unicomb et al. 2005, McCallum et al. 2013, Paine et al. 2014). Sprouts have been implicated in recent New Zealand salmonellosis outbreaks (Pattis et al. 2022).

Environment: *Salmonella* present in sewage effluents or animal faeces can contaminate pasture, soil and water. These bacteria do not usually multiply in soil and waters (this will depend on other growth factors and conditions present) but may survive for long periods, especially in dry environments (Bell and Kyriakides 2002, Haysom and Sharp 2003). *Salmonella* has been detected in surface waters in New Zealand (Till et al. 2008, Leonard et al. 2020). Bacteria can be dispersed in dust and aerosols generated during animal handling and processing. Contamination in the environment can be spread by wind, water and wildlife.

Transmission routes: *Salmonella* may be transmitted via person-to-person transmission, contaminated food or water, animal contact or from a contaminated environment. A review of non-typhoidal salmonellosis sporadic cases and outbreaks in New Zealand indicated that the important pathways for *Salmonella* infection are consumption of contaminated food, consumption of untreated drinking water and contact with sick animals (King et al. 2011a).

2.2 THE FOOD: CHICKEN EGGS

Key findings

- Whole eggs inhibit bacterial contamination of the contents through physical barriers (cuticle, shell, membranes) and antimicrobial components in the albumen. However, the egg yolk is high in nutrients and supports bacterial growth.
- The annual number of eggs produced in New Zealand has fluctuated since the 2016 Risk Profile. In 2022, there were approximately 1.2 billion chicken eggs produced from a per-month average of 3.63 million layer hens. This is similar to numbers from 2015, when the national flock of layer hens was estimated at 3.48 million birds, producing approximately 1 billion eggs. However, production was higher in intervening years, with as high as approximately 4.2 million birds in lay in April 2020. A new Code of Welfare requiring phased prohibition on the use of conventional cages by the end of 2022, together with a commitment by some supermarkets to only sell cage-free eggs by the end of 2025, has impacted egg production recently.
- The majority of eggs are sold as fresh, whole eggs in New Zealand but liquid and dried egg products are also available.
- Regarding international trade, there has been a 44% reduction in the export of eggs and egg products from 2015 (1,970 tonnes) to 2022 (1,100 tonnes). Exports consisted mostly of whole, fresh eggs. The amount of egg products imported into New Zealand has almost tripled since 2015, increasing from 346 tonnes in 2015 to 983 tonnes in 2022. The largest proportion of imported egg products were dried eggs. Fresh eggs are not imported.

2.2.1 Eggs

Eggs are a popular food not only for their nutritional aspects, but also for their functional properties; for example, the coagulant capacity of proteins, the foaming capacity of albumen proteins and the emulsifying capacity of the yolk (European Food Safety Authority and European Centre for Disease Prevention Control 2015). These properties are used in different ways to produce and enrich many types of foods, for example, pastries, sauces, dressings, desserts and pasta. Eggs are often consumed raw or only lightly heat-treated (Section 2.5.5). As reported in the 2016 Risk Profile (Rivas et al. 2016), the majority of eggs are marketed and consumed as fresh shell eggs but liquid eggs and dried egg are also available in New Zealand (Section 2.2.2).

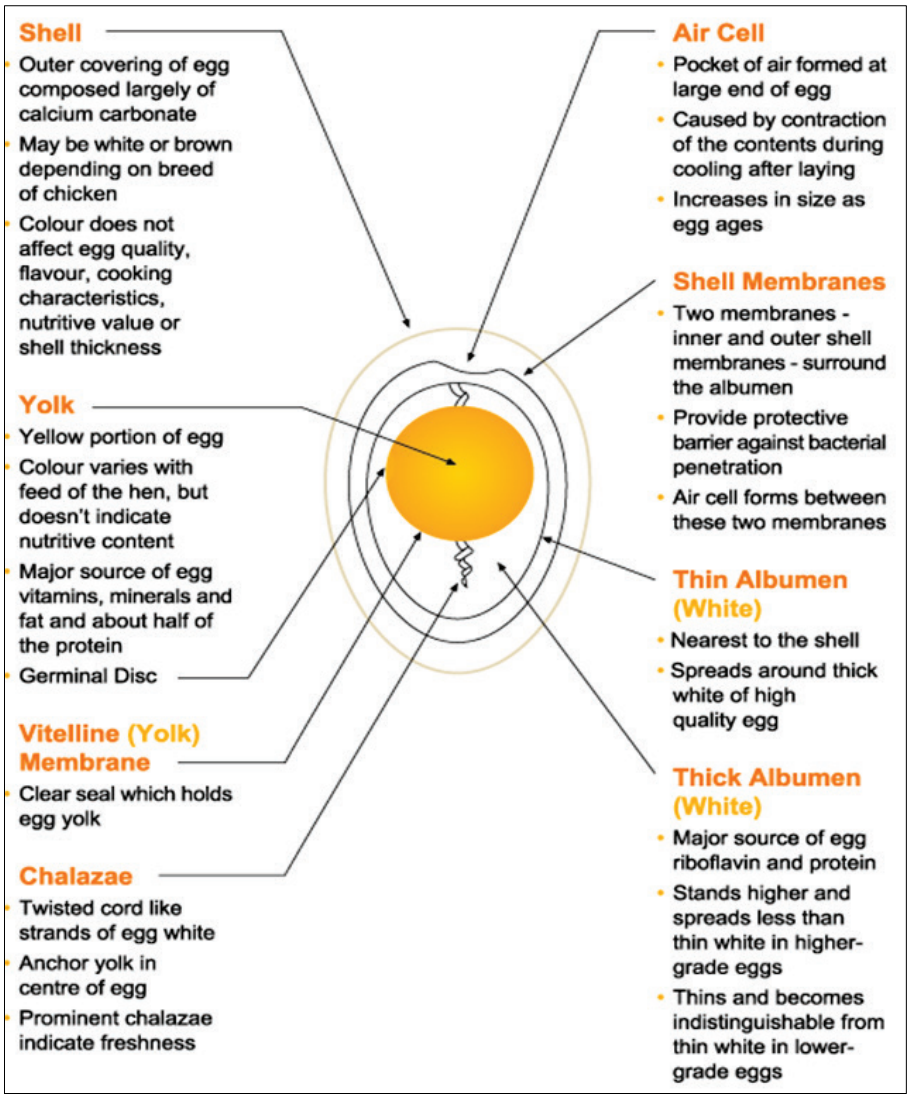

Figure 1. Anatomy of an egg.[1]

[1]Image reproduced with permission from Eggs Incorporated (New Zealand) and Egg Producers Federation of New Zealand (EPF). Image available at http://www.eggs.org.nz/whats-in-an-egg/; accessed 7 March 2023.

Figure 1 shows the major components of an egg. The egg yolk is a nutritious medium for bacterial growth. However, the cuticle, shell and associated membranes create physical barriers to obstruct bacterial contamination of the egg contents, and antimicrobial components present in the albumen (egg white) inhibit bacteria growth (Howard et al. 2012, Baron et al. 2016). Barriers and antimicrobial components include:

- Physical barriers: The cuticle is a protein layer on the exterior of the shell that seals the pores in the calcium-based shell (the cuticle is not shown in the diagram). The cuticle helps to prevent bacteria from getting inside the shell and reduces moisture loss, but is largely removed by abrasion within 96 hours of laying and is also removed by wiping or washing eggs. The shell is the second barrier but this is filled with spiralling pores that penetrate from the outside to the inside, and rapid cooling will cause the internal contents to contract and draw air and/or moisture (and any microorganisms on the eggshell) into the egg. Two membranes under the shell together provide the third outer barrier surrounding the albumen. The vitelline membrane surrounds the yolk and acts as the final barrier between

invading bacteria and the nutrient-rich yolk. Although it is difficult for bacteria to transverse across an intact, good quality eggshell, small defects in the shell such as hairline cracks increase the opportunity for bacteria to penetrate and move into the egg contents (Samiullah et al. 2013).

- Antimicrobial components of the albumen: These include the hydrolase lysozyme, the iron-chelator ovotransferrin, vitamin-chelating proteins such as the biotin-chelator avidin, as well as a number of proteinase inhibitors such as ovomucoid, and defensins (Baron et al. 2016). The concentration of lysozyme and ovotransferrin increase with the hen's age (Gantois et al. 2009). The albumen pH also changes during storage, often reaching pH 9 or greater, which is inhibitory to *Salmonella* growth (Baron et al. 2016).

2.2.2 Primary production of eggs in New Zealand

Poultry is a descriptive term for domesticated breeds of birds including chickens, turkeys, ducks, geese, guinea fowl and quail that are farmed for their meat and eggs.[3] In New Zealand, chickens are by far the largest group of farmed birds; the two main breeds for egg laying are Hyline Brown or Brown Shaver. The term "layer" refers to a chicken which has been bred specifically to produce non-fertile eggs for consumption (table eggs) (compared with the term "broiler" which is often used for a chicken that is bred specifically for meat production).[4] A small number of enterprises farm quail (two farms) or ducks (one farm) for commercial egg production. Data referenced in this section on egg production in New Zealand has been kindly provided by Egg Producers Federation of New Zealand Inc. (EPF; Kerry Mulqueen and Carol Coutts, pers. comm, February 2023).

Relatively small numbers of eggs are produced from small flocks of chickens (including specialty breeds), bantams and ducks kept by people in towns or on farms or lifestyle blocks. The exact number is not known because operations with fewer than 100 birds are not required to register their flocks. However, data from the 2020 Companion Animals New Zealand survey estimate that about 6% of the 1.8 million households in New Zealand own an average of 5.2 birds, and 30% of the birds were chickens (Companion Animals in New Zealand 2020). A major marketing channel for acquisition of backyard poultry is the online trading website, TradeMe®, with an average of 19,610 trades made annually involving 23,768 birds to and from 8,460 traders (Greening et al. 2021). Some of these eggs may be sold to the public, for example, through farmers markets, roadside stalls or the internet.

This Risk Profile has focussed on commercially produced eggs from chickens (*Gallus gallus*). We acknowledge that eggs from other poultry species listed above can also present a risk of salmonellosis to consumers. Other product types have not been considered due to time constraints and the minor contribution to the market share.

EPF represents the interests of all commercial chicken egg producers in New Zealand (but not duck or quail egg producers).[3] Membership is mandatory for egg layer farmers under the Commodity Levies (Eggs) Order 2022.[5] Roles of EPF include research and development,

[3] https://www.eggfarmers.org.nz/; accessed 6 January 2023
[4] https://www.legislation.govt.nz/regulation/public/2022/0252/latest/LMS744523.html?search=ts_act%40bill%40regulation%40deemedreg_animal+products+regulations_resel_25_y&p=1; accessed 28 February 2023
[5] https://legislation.govt.nz/regulation/public/2016/0210/19.0/whole.html; accessed 7 February 2023

technical training and compliance support to help ensure members meet animal welfare, food safety and biosecurity regulations. The EPF liaises with government departments, providing representation on or before boards, committees and commissions constituted under Acts or Regulations applicable to their membership. They also provide funding for the promotion of eggs via Eggs Inc.[6]

The 2016 Risk Profile reported that as at 30 June 2015, the national flock of layer hens was estimated at 3.48 million birds, producing approximately 1 billion eggs annually. Data from EPF indicates that production numbers were similar in 2022 with 1.2 billion chicken eggs produced from a per-month average of approximately 3.63 million birds. However, note that 2015 and 2022 data for birds in lay (and hence, egg production) were lower than data for intervening years, with numbers as high as approximately 4.2 million birds in lay during April 2020 (Figure 2).

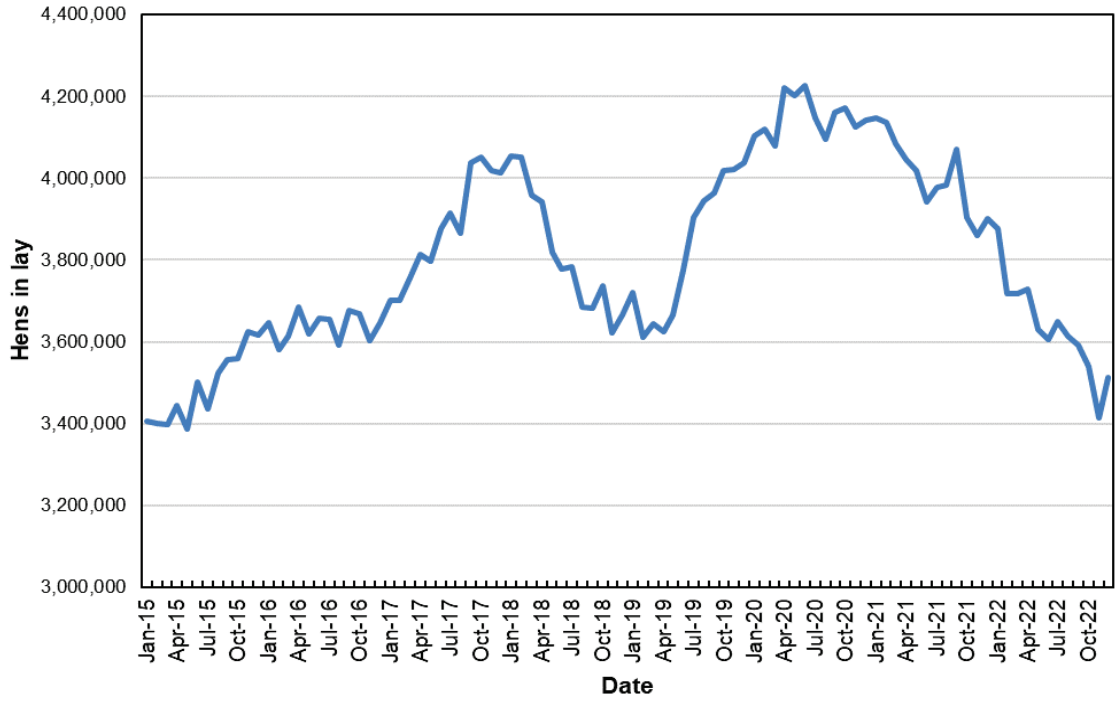

Figure 2. Number of hens in lay (2015 to 2022). Data supplied by EPF.

At least 90% of commercially farmed eggs are sold as 'table eggs', and approximately 5% of the remainder are sold to the consumer as liquid/pulped/pasteurised eggs for human consumption, which are used in the baking and catering industries. In addition, surplus eggs from breeder farms may also be processed to make egg product; the number of which depends on the chick supply, but has been estimated at approximately 200,000 eggs per year.

Primary production of breeder flocks is managed by a small number of companies globally. Grandparent flocks are selectively bred from elite flocks based on precise genetic criteria, such as productivity, quality of products and resistance to disease. Fertilised hatching eggs are then distributed worldwide. The resulting Grandparent chicks are hatched and reared in quarantine facilities, and then transported to parent breeding farms, which ultimately give rise

[6] https://eggs.org.nz/; accessed 7 February 2023

to layer chicks (European Food Safety Authority Panel on Biological Hazards 2019). According to data from EPF, in January 2023 there were 10 breeder farms for layer chickens and four layer hatcheries in New Zealand.

Day-old chicks (defined as chicks up to 72 hours of age which are surviving on their internal yolk sack) are transported from hatcheries to rearer farms where they are raised until the point-of-lay (approximately 18 weeks of age) (Ministry for Primary Industries 2018b). Young layer birds are referred to as chicks from hatching to seven weeks of age, and then as pullets from seven weeks to point-of-lay. As of January 2023, there were 32 rearer farms operating in New Zealand. Rearing may also occur on layer farms.

Upon reaching point-of-lay, chickens are placed in laying facilities. As of January 2023, there were 171 egg layer farms in New Zealand. Laying sheds hold from 100 to 50,000 birds. A December 2016 postal survey that collected demographic information from 33 of the 169 New Zealand commercial layer farms operating at the time reported that there was a median of three sheds per farm (ranging from one to 14) (Greening et al. 2020). The median number of birds reported per layer farm was 8,750 (range from 20 to 150,000).

The different types of housing systems for layer chickens are as follows:

- Conventional cage: an enclosure constructed of metal or plastic and holding 3-7 hens. Cages do not have perches and/or nest areas. They are inside a building and can be multi-tiered. These have been phased out in New Zealand (see below).
- Colony cage: modified and enlarged enclosure with more space than conventional cages and with perching, nesting and scratching areas. Colony cages may also be referred to as furnished or enriched cages.
- Barn: A building that houses layer hens, with or without access to an outdoor area but with areas for nesting, perching and scratching. Barns with outdoor access are usually referred to as free range and the building can be either fixed or moveable such as a shed, aviary, perchery or ark. If a barn has multiple internal levels it is often referred to as an aviary and/or multi-tier system. Aviary systems provide access to nests and perches at a number of heights or have multiple tiers which consist of a raised slatted area with perches and access to food/water at each level.
- Free-range: The key difference between barn-raised and free-range housing systems is that free range birds have access to the outdoors. In larger farms, flocks are housed in sheds fitted with nest boxes and perches, and birds are able to access the outdoors through pop-holes in the shed walls.[7]

As at December 2022, 10% of the national layer flock were housed in conventional cages, 33% in colony cages, 24% in barn and 33% were free range.[8] This compares with 67.1% in conventional cages in December 2016 and 86% in 2012. The main driver for the shift in housing systems is the phased prohibition on the use of conventional cages under Regulation 21 of the Animal Welfare (Care and Procedures) Regulations 2018.[9] Phasing was dependent on the age of the cage system; the final date for the phased prohibition was 1 January

[7] https://www.eggfarmers.org.nz/egg-farming-in-nz/farming-types/free-range; accessed 6 February 2023
[8] https://www.eggfarmers.org.nz/egg-farming-in-nz/farming-types/free-range; accessed 6 February 2023
[9] https://www.legislation.govt.nz/regulation/public/2018/0050/latest/LMS22854.html?search=sw_096be8ed81ac25f0_cage_25_se&p=1&sr=2; accessed 28 February 2023

2023. Housing systems must now meet the requirements for allowing hens the opportunity to express a range of ancestral behaviours, which include: nesting, perching, scratching, ground pecking and dustbathing. These standards and specifications are detailed in the *Code of Welfare: Layer Hens (2018)* (Ministry for Primary Industries 2018b).

A further contributor to the shift in layer housing systems has been the 2017 announcement that some supermarkets have committed to selling only cage-free eggs by the end of 2024 in the North Island, and the end of 2025 in the South Island.[10] The ban on cage-eggs (which includes colony-cage eggs) combined with the phasing out of conventional cages saw a national shortage of eggs available in New Zealand in December 2022.[11]

2.2.3 International trade

New Zealand has a small egg-related export base to the Oceania region, Hong Kong and Singapore.[12] Poultry export data for the period 2015 to 2022 were extracted from Statistics New Zealand[13] and collated in Figure 3. For this period, the main export destinations were Hong Kong (2,505 tonnes; 23.1% of egg exports), Singapore (2,353 tonnes; 21.7% of egg exports) and New Caledonia (2,294 tonnes; 21.2% of egg exports). The majority of exported product was whole eggs (predominantly, fresh eggs from chickens, but also a small amount of fresh eggs from other poultry types, and eggs in shell that had been preserved or cooked) (Figure 3). There were also small amounts of egg products (mainly the category "cooked by steaming or boiling in water, moulded, frozen or otherwise preserved"). There has been a 44% reduction in the weight of whole eggs and egg products exported from 2015 (1,970 tonnes) to 2022 (1,100 tonnes).

Previously, the absence of *S*. Enteritidis in New Zealand poultry provided a market access advantage for poultry export markets (Ministry for Primary Industries 2022i). After the detection of *S*. Enteritidis in commercial egg layer flocks in 2021, *S*. Enteritidis export legislation was introduced in July 2021 as part of a wider risk management programme (Section 4.1).

The only raw eggs that can be imported into New Zealand are fertilised eggs for hatching, and those deemed "Specific-Pathogen-Free" (Ministry for Primary Industries 2022g). The import of fresh table eggs into New Zealand is not permitted. Requirements for the import of egg products are specified in the Import Health Standard: Egg Products (Ministry for Primary Industries 2019b). The amount of egg products imported into New Zealand has almost tripled since 2015, increasing from 346 tonnes in 2015 to 983 tonnes in 2022 (Figure 3). Whole eggs only made up a small proportion of the total weight of imported egg products (15.0% by weight of all egg imports from 2015 to 2022) and all of these were preserved or cooked. The largest proportion of imported egg contents were dried eggs (54.7% by weight of all egg imports from 2015 to 2022), and the remainder comprised of dried yolks only, liquid contents, or contents that had been cooked, frozen or preserved. The main countries of origin for egg products for the years 2015 to 2022 were Italy (2,676 tonnes; 48.0% of all imported egg product), the

[10] https://www.countdown.co.nz/info/community-and-environment/environmental-sustainability/responsible-and-sustainable-sourcing/path-to-cage-free; accessed 28 February 2023
[11] https://www.rnz.co.nz/news/national/481515/egg-shortage-supermarket-shelves-bare-as-industry-deals-with-supply-issues; accessed 3 March 2023
[12] https://www.eggfarmers.org.nz/egg-farming-in-nz; accessed 6 February 2023
[13] https://infoshare.stats.govt.nz/; accessed 3 March 2023

People's Republic of China (1,059 tonnes; 19.0% of all imported egg product), and Australia (937 tonnes; 16.8% of all imported egg product).

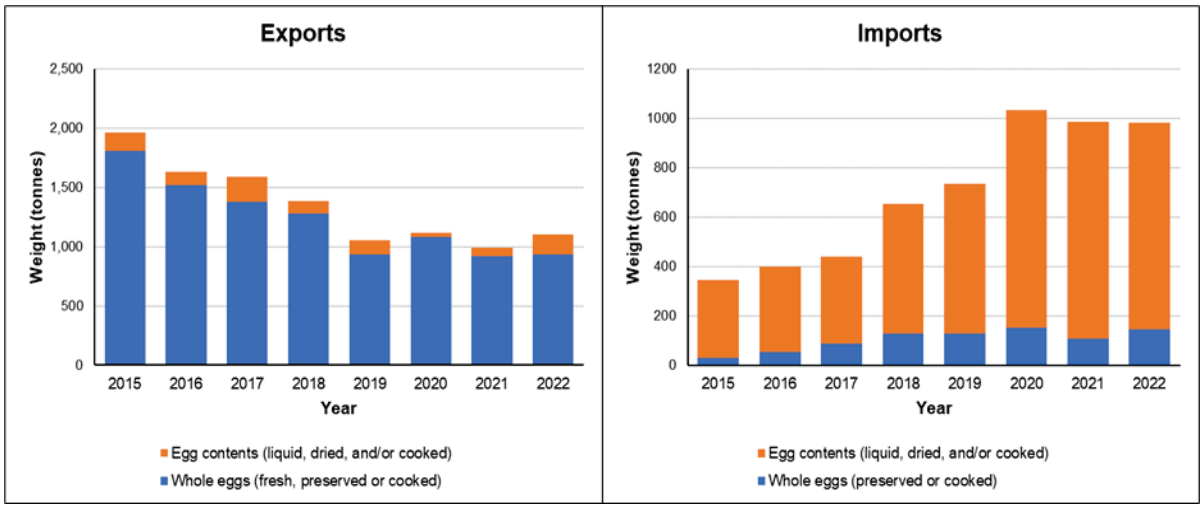

Figure 3. Weight of whole eggs (fresh, preserved or cooked) and egg contents (liquid, dried and/or cooked) exported into and imported from New Zealand per year from 2015 to 2022.[1]

[1]Data extracted from Statistics New Zealand Infoshare using Harmonised Trade codes (http://www.stats.govt.nz/infoshare/, accessed 3 March 2023). Export data does not include export of fertilised eggs.

2.3 *SALMONELLA* COLONISATION OF LAYING HENS AND CONTAMINATION OF EGGS

Key findings

- Layer chickens can be exposed to *Salmonella* from a range of sources from the external environment (for example, other livestock and wildlife and workers), poultry feed and water, and the poultry housing environment (for example, the previous flock or other current flocks if multi-age, insects and rodents, litter).
- The influence of housing system on *Salmonella* prevalence in layer flocks and on eggs is an important consideration given the increasing percentage of eggs produced in New Zealand from cage-free flocks. A 2019 survey of *Salmonella* in New Zealand layer farms found a significantly lower prevalence in the layer environment from cage-free (free range and barn) compared with caged systems (conventional and colony cage). However, there are multiple interconnected practices that differ between housing systems that might also contribute to *Salmonella* prevalence, such as flock density, flock size, multi-age management and bird stress, which are all more common in caged systems. There are conflicting findings on the influence of the housing system to *Salmonella* prevalence from other international studies.
- Once ingested by a bird, *Salmonella* colonises the host intestinal tract and are excreted with faeces, exposing other birds in the flock. Faecal-oral transmission is the primary route by which *Salmonella* colonise chickens. The gut of a chicken may remain colonised with *Salmonella* throughout the bird's lifespan.
- Colonised chickens can contaminate the egg surface (all serotypes) and egg contents. Contamination of egg contents can occur through *Salmonella* migrating through the

eggshell into the contents (all serotypes), or by transovarian transmission. This is the movement of *Salmonella* from the ovaries into the egg contents before full egg formation, and is more likely for invasive strains such as *S*. Enteritidis.

- Contaminated eggs from layer chickens pose a risk to consumers, while contaminated eggs from breeder chickens pose a risk to unhatched and newly hatched chicks that can become colonised by *Salmonella*.
- The serotype *S*. Enteritidis is an invasive serotype that can move from a bird's intestine to colonise their reproductive tract, posing a risk for transovarian transmission. Strains of some *S*. Enteritidis phage types such as DT8 have been reported to have this ability, although it is not known how conserved transovarian transmission is for all *S*. Enteritidis strains. The genetic determinants for what makes a strain capable of transovarian contamination of eggs are not well understood.
- The first detection of *S*. Enteritidis ST11, DT8 in New Zealand poultry was from a processed poultry carcass during 2021, followed by detection in hatcheries and poultry sheds (from both layer and broiler flocks) from which the birds associated with the carcass meat detection were originally sourced. It is not known whether the *S*. Enteritidis strain identified in New Zealand poultry is capable of transovarian transmission to eggs, or whether the strain is more invasive in chickens than other serotypes present in New Zealand layer flocks. The strain has not been detected in eggs from colonised flocks, but minimal testing of eggs has been conducted.

The following information is a summary of activities that influence the introduction, growth or elimination of *Salmonella* along the egg production chain.

2.3.1 *Salmonella* colonisation of poultry

The primary route of infection and transmission of *Salmonella* in chickens is faecal-oral. Once ingested, *Salmonella* initially colonises the intestinal tract of poultry. While colonisation of poultry is usually asymptomatic, it can lead to illness and death in chicks younger than two weeks old (Dunkley et al. 2009). Within a few days of colonisation, chickens excrete *Salmonella* in faeces, at numbers which can be as high as 9 $\log_{10}$ colony forming units (CFU)/g (Thippareddi et al. 2022). Due to the coprophagic (faeces-eating) behaviour of chickens, *Salmonella* can quickly spread from colonised to non-colonised birds in a flock (Gast et al. 2014, Thippareddi and Singh 2022). Furthermore, *Salmonella* can survive in dust in the laying shed environment for a long period of time, and recolonisation of chickens can occur.

The frequency and level of *Salmonella* shedding may vary over time depending on the bird age at the time of infection, the *Salmonella* serotype and exposure dose (Gast et al. 2011, Schulz et al. 2011). In one study, chickens exposed to *S*. Enteritidis shortly after hatching remained colonised on reaching maturity and were still shedding *Salmonella* in faeces at 24 weeks (Gast and Holt 1998). Intestinal colonisation typically declines steadily during the initial weeks after experimental infection. Some birds can clear the infection after three weeks (Beal et al. 2004, Beal et al. 2006). In a study of mature laying hens (31 or 41 weeks of age) that were experimentally inoculated with *S*. Enteritidis, faecal shedding was detected for up to 10 weeks post-inoculation (Gast et al. 2017a). In another study, chickens inoculated with *S.* Heidelberg shed *Salmonella* in their faeces for at least eight weeks, while those inoculated with *S*. Typhimurium ceased shedding by five weeks or more (Gast et al. 2017b). In a further study, a layer flock was inoculated with *S*. Typhimurium at 18 weeks of age and monitored for

40 weeks post-inoculation, which is a longer period than in most studies (McWhorter and Chousalkar 2018). Although faecal shedding varied over time, 60% of faecal samples were still *Salmonella*-positive after 40 weeks.

Peak shedding of *Salmonella* by colonised poultry usually occurs during periods of stress, which can suppress the immune system. Shedding occurs either from reactivation of infection in latent carriers or due to a higher susceptibility of stressed birds to re-infection from the environment (European Food Safety Authority (EFSA) Panel on Biological Hazards (BIOHAZ) 2014). The increased shedding can lead to an increase in the percentage of a flock colonised. Examples of stress events include onset of lay, water deprivation, viral or coccidial infection, stressful environments and moulting (Wigley et al. 2005, European Food Safety Authority Panel on Biological Hazards 2014, Gole et al. 2014a, Crabb et al. 2019b). Note that induced moulting is not permitted in New Zealand commercial layer flocks (Ministry for Primary Industries 2018b).

Some *Salmonella* serotypes have been shown to cause systemic infection of chicken internal organs. These invasive serotypes can colonise the intestinal tract, get taken up by dendritic cells and macrophages and be transported via the bloodstream and lymphatic system to different organs such as the liver, spleen and bone marrow. This can occur within a few hours after oral exposure (Chappell et al. 2009, Gantois et al. 2009, He et al. 2010, Mastroeni and Grant 2011). For *S*. Enteritidis, systemic dissemination of infection in mature hens can reach the reproductive organs (Section 2.3.2).

Hatchery chicks can become colonised by *Salmonella* present on eggshell surfaces, either arising from colonised breeder birds during laying or from a contaminated environment (for example, the nest box, the hatchery environment or the hatchery truck). Newly hatched chicks have an immature intestinal flora and immune system, which makes them more susceptible to *Salmonella* colonisation than older birds (Gast and Beard 1989, Cox et al. 1990). The colonising dose is very low for chicks within the first few days following hatching, then progressively increases with age as the immune system and intestinal flora matures (European Food Safety Authority Panel on Biological Hazards 2019). There is also evidence of increased *Salmonella* persistence when birds are infected at a younger age (Beal et al. 2004). Hatchery chicks can also become colonised by *Salmonella* before or after hatching, when the egg contents have become contaminated with *Salmonella*.

2.3.2 *Salmonella* contamination in and on eggs

Salmonella can contaminate the surface of eggs (external contamination) or the egg contents (internal contamination). An egg contaminated by *Salmonella* will not necessarily look, feel, taste, or smell differently to an uncontaminated egg. External contamination of the shell during laying may arise from *Salmonella* colonising the lower reproductive tract or gastrointestinal tract. Shell contamination may also occur from the environment into which the eggs are laid. External contamination of eggshells presents a risk to humans either directly through contamination of hands and utensils by *Salmonella*, or by the introduction of *Salmonella* into foods when breaking eggs. *Salmonella* contamination of eggshell surfaces is more common than contamination of egg contents both in New Zealand and internationally (Table 13, Appendix) (Rivas et al. 2016, Kingsbury and Soboleva 2019a).

Contamination of internal egg contents can occur by two mechanisms (Gantois et al. 2009, Ministry for Primary Industries 2015, Rivas et al. 2016, Shah et al. 2017, Kingsbury and Soboleva 2019a). These are:

- Transovarian (vertical) transmission: Where *Salmonella* causes systemic infection in a hen and colonises the reproductive organs, where it can be incorporated into the yolk, albumen, eggshell membranes or shell before the egg is laid; and
- Trans-shell (horizontal) transmission: Where *Salmonella* penetrates the eggshell and membrane and reaches the egg contents. The *Salmonella* might originate from the colonised hen gut or from faeces that comes into contact with the egg during or after laying.

S. Enteritidis is still considered to be the most common serotype capable of transovarian transmission, but other *Salmonella* serotypes can infect reproductive tissues, including *S*. Typhimurium and *S*. Heidelberg (Wales and Davies 2011, Martelli and Davies 2012, European Food Safety Authority Panel on Biological Hazards 2014). Experiments have demonstrated that colonisation of the reproductive organs can result in *S*. Enteritidis becoming incorporated into the contents of eggs before they are laid (transovarian transmission) (Gast et al. 2013b, Gast et al. 2016, Gast et al. 2019). This transovarian transmission is important for layer flocks in terms of the risk this poses to human health from internally-contaminated eggs. However, as signalled above, this pathway can also lead to the colonisation of hatchery chicks.

Previously, certain phage types of *S*. Enteritidis were thought to be more capable than others of transovarian transmission; particularly DT4 and DT8, but this capability has also been reported for DT28, DT104, DT13 and DT13a (Thiagarajan et al. 1994, Gantois et al. 2009, Shah et al. 2017). However, strains of a single phage type are not necessarily closely genetically related and conversely, genetically related strains may not be of the same phage type (Section 2.1.1). The extent by which transovarian transmission is conserved for all *S*. Enteritidis phage types, sequence types or strains is not known. The genetic determinants for transovarian transmission are currently not well defined (Gantois et al. 2009, Shah et al. 2017). Studies have focussed on the role in gastrointestinal invasion of the type-3 secretion system (T3SS) encoded by *Salmonella* pathogenicity islands, and flagella and fimbriae factors (Shah et al. 2017). There are some data indicating that type-1 fimbriae and lipopolysaccharide synthesis genes contribute to reproductive tract pathogenesis.

Prior to 2021, transovarian transmission to chicks and to table eggs was not of concern in New Zealand because *S*. Enteritidis had not been detected in New Zealand poultry. However, an incursion of *S*. Enteritidis DT8, ST11 was detected in 2021 (Section 3.3.6). To date, this outbreak strain has not been detected in the contents of eggs from infected flocks, but only minimal testing of eggs has been conducted. In addition, the strain has not been determined to be capable of colonising the chicken reproductive tract or of transovarian transmission to eggs, but this has not been tested.

Genetically, *S*. Enteritidis forms a closely related cluster with the avian serotypes *S.* Pullorum and *S.* Gallinarum, which cause serious infections for poultry (pullorum disease and fowl typhoid, respectively) (Thomson et al. 2008, Luo et al. 2021). While some human cases infected with these serotypes have been reported, these serotypes are usually host-specific and of no major concern for human salmonellosis (Shivaprasad 2000, Uzzau et al. 2000). *S.*

Gallinarum is not present in New Zealand and *S*. Pullorum has not been detected in New Zealand since 1985 as shown by serological monitoring of commercial breeder flocks (Egg Producers Federation of New Zealand 2002).

Whereas transovarian transmission is demonstrated for *S*. Enteritidis, a wider variety of serotypes of *Salmonella* are potentially capable of trans-shell transmission (Gantois et al. 2009, European Food Safety Authority Panel on Biological Hazards 2014). However, there is evidence that *S*. Enteritidis may survive better in the egg albumen than some other serotypes (Gantois et al. 2009, Shah et al. 2017, Kingsbury and Soboleva 2019a). While microbial growth is limited in the albumen, any *Salmonella* cell reaching the nutrient-rich yolk has the potential to grow to high levels depending on the temperature. The behaviour of *Salmonella* in and on eggs is described in more detail in Section 2.4.

It has been suggested that poor shell quality increases the opportunity for *Salmonella* to penetrate eggs. As birds age, they generally produce eggs with poorer scores on shell quality measures and it has been found that eggs from caged flocks scored better on the shell and internal egg quality variables than those from free range flocks (Roberts et al. 2013, Samiullah et al. 2013). However, other studies have suggested no relationship between shell quality and internal contamination by *Salmonella* (Rathgeber et al. 2013). Older flocks are a risk factor for *Salmonella* contamination of eggs but this may also be a result of *Salmonella* colonising and remaining persistent within the flock housing, and circulating in the flock.

Microcracks are small cracks that are not observable by normal candling (using a bright light source behind the egg to show details through the shell) or by the various equipment used to detect cracks in eggs. The presence of microcracks in the shell and the absence of the cuticle has been reported to increase the probability of trans-shell penetration by *Salmonella* (RESCAPE 2009). This suggests that the absence of visible cracks is not a guarantee of shell integrity.

How the eggs are handled or treated throughout the production chain (extrinsic factors, such as washing or wiping) can affect the integrity of the intrinsic factors that protect the egg from bacterial penetration and growth inside the egg (Ministry for Primary Industries 2015). This is further discussed in Section 2.4.

2.3.3 Risk factors for *Salmonella* colonisation of flocks and contamination of eggs along the production chain

Colonisation of layer flocks and a contaminated layer environment are interconnected and both present a risk for eggs contamination. Therefore, controlling the entry of *Salmonella* into the poultry house and colonisation of the bird gut is important for minimising the risk of *Salmonella* from eggs. *Salmonella* can enter a layer flock and spread in the flock environment via a range of vectors. There are different risk factors for *Salmonella* colonisation associated with the different points in the production chain. The point of contamination in the supply chain affects the risk of further dissemination through the supply chain.

Breeder farm: Theoretically, a single elite female bird can give rise to 20-40 grandparent birds, which ultimately give rise to ~300,000 laying hens producing up to 9.0 x 10^7 table eggs

(European Food Safety Authority Panel on Biological Hazards 2019). Given this placement at the apex of the supply chain, a contamination event poses the greatest risk of dissemination through the supply chain. Therefore, stringent measures are essential on breeder farms to ensure that *Salmonella* and other diseases are not amplified through the supply chain via external or internal contamination of hatching eggs. In New Zealand, these measures currently include strict biosecurity measures with regards to personnel, flock movement, plus intensive environmental testing for *S*. Enteritidis (Section 4.1) (Ministry for Primary Industries 2022c).

Despite stringent controls in place for current primary breeding facilities, international trade of infected breeding stocks may have caused the global spread of *S*. Enteritidis in the 1980s and 1990s, as well as more recent incursions into some countries. Evidence for this comes from an assessment of the genetic relatedness of *S*. Enteritidis from different continents, together with information on the international trade networks of breeding stock (Li et al. 2021).

Hatchery: Fertilised, laid eggs are transported to incubators in hatcheries, which is the first location during primary production where colonisation of the layer chickens can occur. *Salmonella* colonisation of unhatched chicks may occur if *Salmonella* was present in egg contents, and hatched chicks may become colonised from contaminated egg shells or contents.

A risk factor analysis of Great Britain broiler hatcheries (Withenshaw et al. 2021) found that out of 64 risk factor variables investigated, using a closed waste disposal system was negatively associated with *Salmonella* detection (odds ratio [OR} 0.08, 95% confidence interval [CI] 0.04−0.18). *Salmonella* detection was positively associated with:

- having ≥30 hatchers in regular use compared to fewer (OR 23.7, CI 6.7–84.2),
- storing trays in process rooms (OR 28.8, CI 7.8–106.3),
- drying set-up trolleys in corridors (OR 15.6, CI 5.9–41.4), and
- having skips located in enclosed areas (OR 8.99, CI 5.89–41.35).

Based on these findings, the authors recommended the following to Great Britain broiler hatcheries (note that the same recommendations would also be valid for layer hatcheries):

- Attention should be paid to the thorough and regular decontamination of egg incubators, hatchers and hatcher areas using effective disinfectants at adequate concentration, including fixtures and fittings that may be more difficult to access.
- Care should be taken to prevent the re-contamination of recently cleaned equipment within all hatchery areas by storing cleaned equipment in separate dedicated rooms.
- Closed waste disposal systems should be used where feasible and staff should be regularly trained in handling waste material to prevent the dissemination of contamination via poor waste handling.
- Thorough and regular cleaning and disinfection of waste areas, including waste egg processing areas, should be included in routine procedures to prevent build-up of contamination in these areas.
- Biosecurity practices should be maintained to a high standard regardless of workforce size, with regular staff training and reinforcement of the importance of high biosecurity by hatchery management.

- Effective monitoring for *Salmonella*, based on regular testing of hatcher debris and macerated waste, should be in place within hatcheries so that hatchery managers are fully aware of the extent of contamination issues.

Because a small number of hatcheries supply all commercial layer flocks in New Zealand, and the same hatcheries also supply broiler chickens, hatcheries are also subject to strict biosecurity controls and frequent environmental monitoring for *S*. Enteritidis (Ministry for Primary Industries 2022c). The key management areas which affect the health and welfare of newly hatched chicks (including areas relevant to *Salmonella* control) are detailed by the *Code of Welfare for Layer Hens (2018)* (Ministry for Primary Industries 2018b), and include:

- cleaning and hygiene
- promptness of removing chicks from hatch machines after hatching,
- grading of day-old chicks,
- destruction of cull chicks and unhatched eggs, and
- holding room conditions.

Rearing and layer farm: The greatest risk to human health along the egg production chain occurs from *Salmonella* colonisation of layer flocks, which has the potential to contaminate eggs. Within the rearing and laying periods, there are a range of different sources and vectors into the shed by which the flock could contract *Salmonella*, including litter, faeces, feed, water, fluff, dust, shavings straw, insects, contaminated equipment, or by contact with other poultry or animals (for example, rodents, wild birds) or workers with contaminated clothing. Processes and practices to minimise *Salmonella* ingress into, and control within sheds are covered in New Zealand by the *Animal Products Notice: Production, Supply and Processing* (see Section 4.1) (Ministry for Primary Industries 2022c). As discussed in the 2016 Risk Profile (Rivas et al. 2016) a systematic review of studies mostly from the EU and US identified a wide range of risk factors for *Salmonella* contamination of shell eggs (Denagamage et al. 2015). Risk factors included:

- High level of manure contamination with *S.* Enteritidis;
- Mid-phase of production (hen age of 35-56 weeks);
- High degree of egg-handling equipment contamination;
- Flock size of >30,000;
- Egg production rate of >96% (percentage of birds in a flock actively laying eggs).
- The presence of previous *Salmonella* infection;
- Absence of cleaning and disinfection;
- Presence of rodents;
- Induced moulting (note that induced moulting is not allowed in New Zealand);
- Multi-age management;
- Cage housing systems;
- In-line egg processing;
- Rearing pullets on the floor;
- Pests with access to feed prior to movement to the feed trough;
- Visitors allowed in the layer houses; and
- Trucks near layer sheds, particularly the air inlets of sheds.

The effect of the flock housing system on *Salmonella* prevalence in the environment and eggs is important given the increasing percentage of eggs now produced in New Zealand from cage-free flocks. Free range production may increase the risk for of chickens being exposed to *Salmonella* in the outdoor environment. Birds raised in free range production systems are also potentially exposed to more environmental stressors than caged birds, including social stress and aggression, predation and thermal challenges (Chousalkar et al. 2018b). However, improved gut heath, which could help prevent or reduce *Salmonella* colonisation, is one of the advantages of free range flocks. Multiple practices associated with caged flocks also influence *Salmonella* prevalence, such as a higher flock density, larger flock size and multi-age management (European Food Safety Authority Panel on Biological Hazards 2019). Increased *Salmonella* prevalence in caged flocks with higher densities has been contributed to diminished immune responses due to higher stress, and increased opportunities for horizontal exposure to *Salmonella* (Gast et al. 2017a). Larger flocks can result in higher levels of *Salmonella*-contaminated dust and dander being produced which can re-infect birds (Denagamage et al. 2015). Multi-age management poses a risk because it is more challenging to clean laying sheds following the depopulation of one flock when birds from another flock still remain in the shed (Mollenhorst et al. 2005, Wales et al. 2007, Huneau-Salaun et al. 2009).

Comparisons of *Salmonella* contamination between free range and "conventional" barn housing systems have produced varied results (Young et al. 2009). Some recent comparisons between free range and caged flocks did not find differences in the prevalence of *Salmonella*-infected flocks (Wierup et al. 2017, Rothrock et al. 2021). However, one of the studies reported that the *Salmonella* serotypes found on free range (and organic) farms were different to those found on "conventional" farms (Rothrock et al. 2021). The serotypes found on free range farms were farm-specific and less commonly reported from human salmonellosis cases. Other reports have also found either no difference between housing systems, or a higher contamination of eggs from free-range systems (Jones et al. 2012, Jones et al. 2015, Parisi et al. 2015, European Food Safety Authority Panel on Biological Hazards 2019)

In contrast, other studies have found a higher *Salmonella* prevalence in environmental samples or eggs from caged systems relative to cage-free sheds (Holt et al. 2011, Jones et al. 2012, Cuttell et al. 2014, Parisi et al. 2015, Neira et al. 2017, Crabb et al. 2019b). Furthermore, a recent EFSA review of risk factors for *Salmonella* in laying hens concluded that the overall evidence supported a lower occurrence in cage-free systems (European Food Safety Authority Panel on Biological Hazards 2019).

A 2016 cross-sectional survey of 28 New Zealand egg layer farms identified a significantly higher prevalence of *Salmonella* in caged sheds (conventional and colony; 33/75 positive sheds, 44.0%; $P < 0.001$) compared with cage-free sheds (barn and free range; 4/126 positive sheds, 3.2%) (Kingsbury et al. 2019a). *Salmonella* prevalence increased with increasing flock size, and was higher in sheds with multi-aged compared with single aged flocks in this survey.

Surveys of eggs also signal that housing system can affect *Salmonella* contamination. In a New Zealand survey, *Salmonella* was only isolated from cage-laid, but not free-range or barn-laid, eggs at retail (Wilson 2007). An Australian study reported different proportions of

Salmonella serotypes from eggs produced by barn and cage production systems (Sodagari et al. 2021). The majority (11/12; 92%) of *S*. Infantis isolates were from barn or cage production systems, whereas *S*. Typhimurium isolates were recovered from free-range, barn and cage eggs. *Salmonella* from cage eggs also had more virulence genes than those from free range systems. In particular, the genes four virulence plasmid genes *spvB*, *spvC*, *sseL* and *phoQ*, which are involved in toxin production and promotion of *Salmonella* survival and growth in the host, were identified almost exclusively from cage and barn eggs. A Canadian study found that compared with conventional cage eggs, free range eggs had better cuticle quality and lower bacterial adherence (Kulshreshtha et al. 2021). However, a survey of Australian eggs found that free range eggs were significantly more likely to be dirty and have rough surfaces compared with cage eggs ($p < 0.05$ for both parameters) (Symes et al. 2016). A lower proportion of free range eggs were cracked (2% compared with 4% for cage-eggs) but these differences were not significant).

Salmonella might also be introduced to eggs during egg collection and at the packhouse from contact with workers or from cross-contamination with egg contact surfaces. A Belgian study reported that *S.* Enteritidis was common on equipment and surfaces in egg packing areas on farms where flocks were infected with this bacterium. The egg-collecting area was highlighted as a reservoir for cross-contamination (Dewaele et al. 2012b). In the study by Kingsbury et al. (2019), *Salmonella*-positive egg contact surfaces at New Zealand packhouses were only identified on the three farms with the highest laying shed prevalence, and isolates were genetically related, suggesting cross-contamination was occurring between the laying shed and packhouse surfaces (Kingsbury et al. 2019a). These results suggest that the laying sheds (and/or hens) were the source of the eggshell contamination which was then transported to packhouse surfaces. Associations between the prevalence and types of *Salmonella* in laying sheds and packing sheds emphasises the importance of good on-farm hygiene controls to minimise *Salmonella* contamination of eggs.

More detail of on-farm risk factors for *Salmonella* colonisation of flocks and contamination of eggs has been provided in Appendix A.3.

2.4 BEHAVIOUR OF *SALMONELLA* IN AND ON EGGS

Key findings

- *Salmonella* can survive on eggs for one month or more at temperatures ranging from 4 to 26˚C, when inoculated at high numbers (5-7 log CFU per egg). *Salmonella* survives better at lower temperatures, but viability decreases over time at all temperatures. A study that inoculated egg surfaces with a cocktail of ten New Zealand egg-associated *Salmonella* isolates of five serotypes, found that *Salmonella* viability declined more rapidly on egg surfaces at the higher storage temperature of 22˚C compared with 15˚C. However, faecal contamination on eggshells increased *Salmonella* survival, emphasising the need to ensure that eggs are sold clean.
- Some *Salmonella* strains have been demonstrated to form a biofilm on egg surfaces. Biofilm formation on eggs was influenced by storage temperature and serotype. However, the relevance and extent by which biofilms are able to form on eggs in the egg production environment, and how this is affected by egg washing procedures, is not known.
- All motile *Salmonella* serotypes are likely capable of penetrating the eggshell and moving into the albumen. Lower temperatures slow the rate of penetration, but do not prevent it. Importantly, no contamination of egg contents was observed in a New Zealand study that inoculated the surfaces of intact eggs with a cocktail of egg-associated *Salmonella* strains, at either 15˚C or 22˚C, regardless of the presence of faeces. Available data suggests that, if it occurs at all, the internalisation of New Zealand non-Enteritidis *Salmonella* isolates into unwashed, uncracked eggs, and their survival for the duration of egg storage, is a rare event.
- *Salmonella* may survive in the albumen, but limited-to-no growth will occur unless yolk is also present. *Salmonella* will grow in the yolk or whole liquid egg if the temperature is ≥7˚C.
- During egg processing, egg washing reduces the number of *Salmonella* present on eggshells, but washing practices must be conducted in a manner that does not damage the shell or facilitate egg internalisation by *Salmonella*. Some processors treat egg surfaces with ultraviolet light (UV), which is expected to inactivate *Salmonella* present on surfaces but not within egg pores or egg contents. Egg oiling may also be performed, which preserves the internal quality of eggs by slowing the loss of water and CO_2, and may obstruct bacterial transit through eggshell pores.
- Pasteurisation regimes recommended for use in New Zealand should inactivate most *Salmonella* present in egg contents, but further validation would provide better assurance.
- In the domestic environment, cooking processes that result in undercooked, runny yolks, will permit the survival of any *Salmonella* present.

2.4.1 *Salmonella* behaviour on the surface of eggs

Cross-contamination from contaminated eggshells during food preparation is thought to be the main risk factor for egg-related salmonellosis, particularly in countries where *S*. Enteritidis is not endemic in layer flocks. The *Salmonella* numbers on eggshells at the time of food preparation depends on *Salmonella* survival and, potentially, growth on the eggshell during storage. The 2016 Risk Profile (Rivas et al. 2016) compiled data from studies that investigated *Salmonella* survival on eggshells (Table 1 from that report); and additional data were compiled by Kingsbury and Soboleva (2019) (Table 6 from that report). Studies included in those reports indicate that overall, *Salmonella* survived better on eggs at low temperatures, although results were not always consistent. Regardless of temperature, *Salmonella* were able to survive on the surface of the eggs for several weeks. In one study, *Salmonella* survived 10 weeks,

although the number of cells inoculated onto the eggs was high (5-7 $\log_{10}$ CFU/egg) (Lublin et al. 2015).

Growth of *Salmonella* on clean eggshell surfaces is not expected to occur. However, chicken faeces on the surface of eggs may have a protective effect and act as a source of nutrients for any *Salmonella* present (Schoeni 1995, Park et al. 2015). New Zealand eggs sold at retail are required to be visibly clean. In MPI's Risk Management Programme (RMP) Template for Harvesting, Candling or Packing Eggs, a dirty egg is defined as "an egg with visible (to the naked eye) foreign matter on the shell surface, which can include yolk, manure or soil" (Ministry for Primary Industries 2020). Among the nine (1.8%) of 514 retail egg surface samples that tested positive for *Salmonella* in a 2007 New Zealand survey, four were evaluated to contain at least one "dirty" egg (obvious contamination of shell with faecal, feather or other organic material) (Wilson 2007).

To provide a better understanding of the risk to New Zealand consumers of *Salmonella* present on egg surfaces, the 2016 Risk Profile (Rivas et al. 2016) signalled that studies were required that used lower concentrations of inoculum, focussed on non-Enteritidis serotypes, and were conducted under storage conditions aligned with what eggs would be subjected to in the New Zealand food chain.

To address this data gap and to inform risk management decisions regarding egg storage times and temperatures with respect to *Salmonella* control in and on New Zealand eggs at retail, a study assessed the survival of New Zealand egg and layer farm-associated *Salmonella* isolates on eggs at storage times and temperatures that were in use in New Zealand (Kingsbury et al. 2019b). At the time of the study, the shelf life options for New Zealand eggs were either: 21 days where the storage/holding temperature may exceed 15˚C; or 35 days if the eggs were stored or held at 15˚C or less. The study inoculated eggshell surfaces with a cocktail of ten *Salmonella* isolates comprising five serotypes, at numbers of ~10^6 CFU/egg. Note that this inoculum concentration was necessary to detect a several log CFU decline in recoverable *Salmonella* on eggs over time and to generate statistically significant data to compare the effect of the different incubation temperatures on *Salmonella* recovery from egg surfaces. Inoculated eggs were incubated at 15˚C (31% relative humidity [RH]) and 22˚C (45% RH). At 0, 21, and 35 days of incubation, eggshells were enumerated for *Salmonella*. The change in *Salmonella* numbers on egg surfaces over time at the different storage temperatures is shown in Table 1. *Salmonella* survived better on eggshells at 15˚C than at 22˚C. Recoverable numbers of *Salmonella* from visibly clean eggshell surfaces declined over time at both storage temperatures and were at, or below, the limit of detection from eggs stored at 22˚C and 45% RH for 35 days. The findings demonstrate that *Salmonella* that might contaminate the egg surfaces during egg production at layer farms will not increase in number after 21 days at either temperature, particularly for eggs stored at 22˚C compared with 15˚C.

Also in this New Zealand study, a subset of eggs was artificially contaminated with sterile chicken faeces prior to *Salmonella* inoculation. Higher numbers of *Salmonella* were recovered from eggshells following incubation at 15˚C (31% RH) compared with 22˚C (45% RH) after both 21 and 35 days of incubation. Significantly higher numbers of viable *Salmonella* were

recovered from eggshells that were experimentally contaminated with chicken faeces compared with those without, particularly from eggs stored at 15˚C and 31% RH for 35 days (2.38 log CFU/egg higher from eggs with faeces). If the increased numbers of *Salmonella* on eggs in the presence of faeces was due to faeces supporting growth, it is counterintuitive that *Salmonella* would be present in higher numbers at 15˚C compared with the higher growth rate expected at 22˚C. Instead, results may reflect a general propensity for *Salmonella* to survive better at lower temperatures in conditions of low water activity (Li et al. 2013). *Salmonella* cells might also be more easily washed off the eggs when they are adhered to faeces rather than to the porous egg surface. The increased survival of *Salmonella* in the presence of faeces was consistent with findings reported by Park et al. (2015). The results emphasise the importance of maintaining and enforcing current regulations that require eggs sold at retail to be visibly clean.

While the number of *Salmonella* recovered from eggshells over time is most likely to be reducing as cells die, migration of cells into the egg contents has also been suggested as a mechanism for reduced numbers (Pasquali et al. 2016). However, as discussed in Section 2.4.3, Kingsbury et al. (2019b) did not detect any *Salmonella* in egg contents. It is possible that the reduction in the number of *Salmonella* on the shell surface over time is partly due to cells migrating into the egg pores where they may be less easily recovered for enumeration, but the extent of this effect has not been established.

Data from other recent studies have been compiled in Table 1. One study compared the survival of *S*. Typhimurium on egg surfaces incubated at 5˚C and 25˚C (76 to 82% RH), for 96 hours (Khan et al. 2021). More than one-log higher CFU/ml were recovered from eggs stored at 5˚C, but the data showed large error bars, and the differences were reported as not significant. Another study compared the survival of *S*. Typhimurium on the shells of washed eggs stored at different temperatures (4˚C, 14˚C, 23˚C and 35˚C) and relative humidities (95%, 70%, 40% and 20%, respectively) for up to four weeks (Whiley et al. 2016). A rapid decline in recoverable *Salmonella* was observed after one week storage at all temperature/humidity combinations, with the least decline observed at 4˚C. However, surprising results were observed after four weeks incubation at all temperatures, where recoverable *Salmonella* numbers were observed to increase back to inoculum levels or higher. The authors attributed this increase to an increased permeability of the eggshell over time allowing for a transfer of nutrients, presumably to the eggshell surface, supporting growth. This explanation would assume that inhibitory components also present in albumen were not also transferring to the eggshell surface. An increase in recoverable *Salmonella* (~2.5-log CFU/eggshell from week 3 to week 4) was even observed at 4˚C, a temperature which does not support the growth of *Salmonella* (Appendix A.1). To our knowledge, this increase following an initial decline in *Salmonella* has not been reported elsewhere. Following 28 days incubation, there was no significant difference in viable *Salmonella* recovered from eggshells between the four incubation temperatures. However, the high level of variability of numbers observed at previous time points makes it difficult to draw firm conclusions from these experiments for any effect of incubation temperature on *S*. Typhimurium survival on eggs.

Table 1. Behaviour of *Salmonella* on the shell surface of eggs (studies published since 2016).

Serotype	Experimental setup	Storage conditions	Change in numbers (log_{10} CFU/eggshell)[1]	Reference
Cocktail: Typhimurium, Infantis, Thompson, Anatum, Mbandaka	5.98 log_{10} CFU (in PBS), applied as 5 x 10 µl spots on egg surface	15˚C, 31% RH: 3 weeks 5 weeks	 ↓2.33 ↓2.70	(Kingsbury et al. 2019b)
		22˚C, 45% RH: 3 weeks 5 weeks	 ↓3.37 ↓3.75	
		15˚C, 31% RH, with faeces: 3 weeks 5 weeks	 ↓1.30 ↓1.07	
		22˚C, 31% RH, with faeces: 3 weeks 5 weeks	 ↓3.28 ↓3.69	
Typhimurium	Unwashed eggs dipped in inoculum (7 log_{10} CFU/ml in PBS); ~4.0 log_{10} CFU per egg	4˚C, 95% RH: 1 week 2 weeks 3 weeks 4 weeks	 ↓~1.00 ↓~1.25 ↓~2.25 NC	(Whiley et al. 2016)
		14˚C, 70% RH: 1 week 2 weeks 3 weeks 4 weeks	 ↓~3.00 NC NC ↑~0.75	
		23˚C, 40% RH: 1 week 2 weeks 3 weeks 4 weeks	 ↓~3.25 ↓~2.0 ↓~3.5 NC	
		35˚C, 20% RH: 1 week 2 weeks 3 weeks 4 weeks	 ↓~2.5 ↓>4.5 ↓~1.75 ↑~0.75	
Typhimurium	Sterilised eggshell coupon dipped in inoculum (5-6 log_{10} CFU/ml in PW), incubated for 24 h, 37˚C to form biofilm.	RT, 50% RH: 5 weeks (no rinse before incubation	 ↓2.55	(Lee et al. 2020)
		5 weeks (rinse before incubation)	↓1.49	
Typhimurium	Eggs dipped in inoculum (6 log_{10} CFU/ml in LB broth);); 6.0 log_{10} CFU/ml per egg.	5˚C, 76 to 82% RH, 4 days	NC	(Khan et al. 2021)
		25˚C, 76 to 82% RH, 4 days	↓1.76	

[1] ↓ = decreased by >0.5 log_{10} CFU relative to starting CFU; ↑ = increased by >0.5 log_{10} relative to starting numbers. Data are reported from text, if provided, or estimated from graphs. NC, no change (change ≤0.5 log10); ND, not detected. Other abbreviations: CFU, colony forming units; PBS, phosphate buffered saline; LB, Luria Bertani broth; PW, peptone water; RT, room temperature; RH, relative humidity.

2.4.2 Biofilm formation on eggs

The ability of *Salmonella* to form biofilms on eggshell surfaces has been demonstrated (Pande et al. 2016). Biofilm is comprised of interacting cells embedded in an extracellular matrix. The matrix is produced by the cells and comprised of curli, fimbriae and cellulose polymers, which

promote linkage between the *Salmonella* cells. The formation of a biofilm allows *Salmonella* to better-survive harsh physical and environmental stressors, contributing to its persistence on a wide range of biotic and abiotic surfaces (Steenackers et al. 2012).

A total of 145 Australian egg layer farm isolates comprising seven serotypes were tested for biofilm-relevant phenotypes, biofilm-relevant gene expression, and biofilm formation in vitro and on eggs (Pande et al. 2016). Phenotypes were tested at both at 22˚C and 37˚C, while biofilm formation on eggs was tested at 22˚C only. Significantly greater biofilm production and biofilm-relevant phenotypes were observed at 22˚C compared with 37˚C. Biofilm formation ability on eggs for different serotypes was ranked as follows: *S*. Anatum > *S*. Worthington > *S*. Agona > *S*. Oranienburg > *S*. Typhimurium > *S*. Mbandaka > *S*. Infantis. The relative expression of biofilm-dependent genes *csg*D and *adr*A gene was significantly higher in eggshell biofilm cells of *S*. Mbandaka and *S*. Oranienburg. Further studies have demonstrated biofilm formation of *S*. Typhimurium on chicken eggs, and of *S*. Enteritidis and *S*. Heidelberg on turkey eggshells; the incubation temperature for both studies was 25˚C (Silva et al. 2019, Lee et al. 2020).

The regulation of genes involved in biofilm formation at different storage-relevant temperatures for *S.* Typhimurium incubated on eggshells and in albumen and yolk has also been investigated (Khan et al. 2021). The *csg* genetic region encodes protein polymers known as curli fimbriae, which are important for cell aggregation, adhesion to surfaces and biofilm formation. The gene *csgB* was upregulated at 5˚C and 25˚C in the yolk, albumen and at 5˚C on the eggshell. Other biofilm-relevant genes, *fimH*, *pefA* and *pefB*, were also upregulated in the yolk at both temperatures, and expression was typically higher on eggshells at 5˚C than 25˚C, where the genes were typically downregulated.

The effect of potential eggshell treatments against *Salmonella* biofilm on eggshells has also been investigated. The enzyme ficin and the sanitiser peracetic acid (PAA) were effective against *S*. Thompson biofilms on eggs (Nahar et al. 2022). Although only PAA was bactericidal, the sequential treatment of ficin followed by PAA improved the activity of PAA, causing the greatest degree of biofilm reduction. The highest concentrations of agents tested included 12.5 units/ml of ficin and 270 ppm PAA, which resulted in a 5.01 log CFU/cm^2 reduction of *S*. Thomson on eggshells.

The ability to form a biofilm on eggs may represent an increased food safety risk, making biofilm-forming strains more difficult to eradicate from eggs, and potentially increasing their on-shell survival. However, the method that experimental studies have induced biofilm formation on eggs has involved immersion in high concentrations of *Salmonella* in growth media, which does not emulate natural contamination scenarios. The relevance and extent by which biofilms are able to form on eggs in the egg production environment, and how this is affected by egg washing procedures, is not known.

2.4.3 The ability of *Salmonella* to penetrate eggs (horizontal transmission)

The 2016 Risk Profile (Rivas et al. 2016) reported that *Salmonella* can penetrate the eggshell and colonise the contents, but its ability to do so is influenced by a number of intrinsic factors relating to the egg and extrinsic factors, for example, how the egg is handled, the external

conditions, and the presence of faeces. The authors also reported that refrigeration temperatures appear to reduce the ability of *Salmonella* to penetrate the eggshell, but these lower temperatures can also enhance penetration if eggs were previously stored at high temperatures and rapidly cooled. New data published since 2016 on egg internalisation by *Salmonella* have been summarised in Table 2.

Studies of eggshell penetration by *Salmonella*, mostly non-Enteritidis serotypes, have found that:

- *The quality of the shell does not strongly influence Salmonella penetration*: *S.* Heidelberg were able to penetrate the shells of eggs from a variety of chicken breeds within 45 hours when stored at 35˚C, although there were differences in the numbers of microorganisms detected in the interior (Rathgeber et al. 2013). Measurements of shell thickness and strength were not related to the rate of cell penetration.
- *Non-Enteritidis serotypes can also penetrate eggshells and washing eggs can, in some cases, aid penetration*: Using agar-filled eggs, several Australian studies demonstrated that *S*. Typhimurium, *S*. Infantis, *S*. Singapore, *S*. Adelaide, *S*. Worthington and *S*. Livingstone were all able to penetrate eggshells of washed and unwashed eggs (Samiullah et al. 2013, Gole et al. 2014b, Gole et al. 2014c). However, *S*. Singapore, *S*. Worthington and *S*. Livingstone were not detected in the internal egg contents when they were inoculated on the outside of normal whole eggs, which suggests that these serotypes may have a limited ability to survive in the albumen. Similarly, *S.* Infantis was only detected in the contents of whole eggs by polymerase chain reaction (PCR). Some of the strains studied were better able to penetrate the shells of washed eggs but in most cases there was no significant difference between washed and unwashed eggs in terms of the number of eggs penetrated, despite the washing steps affecting the cuticle cover. These experiments were all conducted at 20˚C or 37˚C, and the eggs were all stored for 21 days after inoculation.
- *The effect of temperature on the rate of penetration is difficult to predict*: *S*. Infantis was able to penetrate into eggs held at 6˚C and 26˚C, but penetration into the egg contents was first measured after two weeks at 6˚C and four weeks at 26˚C (Lublin et al. 2015). A study using agar filled eggs found penetration by *S*. Infantis of up to 96% and 71% of washed and unwashed eggs respectively after 21 days at 20˚C (Samiullah et al. 2013). The proportion of eggs penetrated was similar at 20˚C and 37˚C. Another study examined egg penetration by two strains of *S.* Typhimurium. Penetration by one strain was significantly higher at 20˚C compared with 37˚C, but temperature had no significant effect on egg penetration by the other strain (Gole et al. 2014b). A further study found significantly higher internalisation of eggs by *S*. Typhimurium at higher temperatures (23˚C and 35˚C compared with 4˚C and 14˚C) following incubation for 4 weeks (Table 2) (Whiley et al. 2016).

The relationship between environmental temperature, relative humidity and eggshell temperature affects the development of condensation on eggs (egg sweating), and the right conditions for condensation are most likely to be found during cold chain distribution. A review of older studies suggests that condensation on the eggs may increase *Salmonella* penetration of the shell (Martelli and Davies 2012). A more recent study investigated the effect of egg condensation on *S*. Enteritidis penetration into egg contents over a six week storage period at

4˚C (Gradl et al. 2017). Eggs were inoculated with 6.4 $\log_{10}$ CFU/egg *S*. Enteritidis, and then condensation was induced by a 17 minute incubation in a 32˚C incubator before refrigerated storage. However, *S*. Enteritidis was not detected from egg contents from sweated or non-sweated eggs at any timepoint (selected data are included in Table 2).

In addition to assessing the survival of New Zealand egg-associated *Salmonella* isolates on egg surfaces (described in Section 2.4.1), Kingsbury et al. (2019b) examined the ability of the strains to internalise from the eggshell surface to egg contents. Egg surfaces were inoculated with ~3 $\log_{10}$ CFU/egg, with or without sterile faeces added, and incubated at either 15˚C or 21˚C. The majority of the albumen and the entire yolk were tested using enrichment to increase the likelihood that any viable *Salmonella* would be detected, if present. As shown in Table 2, there was no detection of *Salmonella* in egg contents (albumen nor yolk) from surface-inoculated eggs stored at either temperature, regardless of the presence of faeces on eggs. Results support that internalisation into unwashed, uncracked eggs, and survival for the duration of egg storage of New Zealand *Salmonella* isolates, if occurring, is a rare event. The data are consistent with previous surveys, none of which detected *Salmonella* in the contents of eggs at retail in New Zealand. One caveat was that the eggs used in the 2019 study were "best-case scenario" in that they were unwashed (thus, their cuticles were intact), visibly spotless (except those with faeces artificially added), not cracked, and shells contained no visible deformities. Therefore, experimental results may not equally apply to all eggs at retail.

Some serotypes of *Salmonella* used in the New Zealand study have been shown in other studies, such as those discussed above, to have the ability to become internalised in eggs. Differences observed between these studies might be due to differences in experimental egg inoculation and *Salmonella* detection methods. Whereas Kingsbury et al. (2019b) employed a more natural inoculation approach of application of *Salmonella* in low inoculation volumes at five sites on the egg, other studies used higher inoculum concentrations and/or inoculated eggs by immersion of the egg into the inoculum (Chousalkar et al. 2010, Samiullah et al. 2013, Gole et al. 2014b, Gole et al. 2014c, Lublin et al. 2015, Whiley et al. 2016). Such an approach might promote *Salmonella* internalisation, particularly if there was a temperature differential between the inoculum and the egg, or if eggs took a long time to dry. Any detection of *Salmonella* in egg contents in the study by Kingsbury et al. (2019) would require both transit across the shell and survival in egg albumen. However, detection of internalisation in other studies was determined using an agar egg technique or PCR, neither of which would require *Salmonella* to survive in the albumen (Chousalkar et al. 2010, Samiullah et al. 2013, Gole et al. 2014b, Gole et al. 2014c).

Table 2. Influence of storage conditions and serotype on the internalisation of *Salmonella* into egg contents.

Serotype	Experimental setup	Storage conditions	Results (detection/egg content)		Reference
Cocktail: Typhimurium, Infantis, Thompson, Anatum, Mbandaka	2.98 log_{10} CFU[1] (in PBS), applied as 5 x 10 µl spots on unwashed egg surface. Yolks and albumen tested separately using enrichment.	15˚C, 31% RH:	Yolk:	Albumen:	(Kingsbury et al. 2019b)
		3 weeks	0/10, 0/10	0/10, 0/10	
		5 weeks	0/10, 0/10	0/10, 0/10	
		22˚C, 45% RH:			
		3 weeks	0/10	0/10	
		5 weeks	0/10	0/10	
		15˚C, 31% RH, with faeces:			
		3 weeks	0/10	0/10	
		5 weeks	0/10	0/10	
		22˚C, 31% RH, with faeces:			
		3 weeks	0/10	0/10	
		5 weeks	0/10	0/10	
Typhimurium	Unwashed eggs dipped in inoculum (~7 log_{10} CFU/ml in PBS); ~4.0 log_{10} CFU/egg. Contents tested by direct plating.	4˚C, 95% RH:			(Whiley et al. 2016)
		1 week	0/12		
		2 weeks	2/12		
		3 weeks	1/12		
		4 weeks	3/12		
		14˚C, 70% RH:			
		1 week	2/12		
		2 weeks	2/12		
		3 weeks	0/12		
		4 weeks	6/12		
		23˚C, 40% RH:			
		1 week	3/12		
		2 weeks	10/12		
		3 weeks	7/12		
		4 weeks	12/12		
		35˚C, 20% RH:			
		1 week	9/12		
		2 weeks	12/12		
		3 weeks	11/12		
		4 weeks	12/12		
Enteritidis	Unwashed eggs inoculated with 25 µl 8 log_{10} CFU/ml; ~6.4 log_{10} CFU/egg, condensation induced by 17 min in 32˚C incubator.	4˚C, non-sweated eggs:			(Gradl et al. 2017)
		1 week	0/35		
		6 weeks	0/36		
		4˚C, sweated eggs:			
		1 week	0/33		
		6 weeks	0/36		

[1] Abbreviations: CFU, colony forming units; PBS; phosphate buffered saline; RH, relative humidity.

2.4.4 *Salmonella* behaviour in albumen

Albumen is an unfavourable medium for bacterial growth and mobility due to its high viscosity, high pH and antimicrobial proteins; those considered most relevant for bacterial inhibition are lysozyme and ovotransferrin (Baron et al. 2016, Rivas et al. 2016, Kingsbury and Soboleva 2019a).

Lysozyme is present at high concentrations (3.5 g/L) (Baron et al. 2016). Lysozyme hydrolyses the glycosidic (1-4) β-linkage between the N-acetylglucosamine and N-acetylmuramic acid residues in Gram-positive bacterial peptidoglycan. Because the peptidoglycan layer is

important for structural integrity of the bacterial cell, its degradation can result in cell swelling and lysis in conditions of low osmotic strength. In contrast, the role of lysozyme in inhibition of *Salmonella* growth in albumen remains unclear. Gram-negative bacteria such as *Salmonella* are generally more resistant to lysis by lysozyme due to the presence of an additional, protective outer membrane, which prevents access of lysozyme to the peptidoglycan layer. Furthermore, no inhibitory effect was observed following incubation of *S*. Enteritidis in pasteurised egg white or growth media with increasing concentrations of lysozyme (Hughey and Johnson 1987, Facon and Skura 1996, Jakočiūnė et al. 2014).

Ovotransferrin is also present at high concentrations in egg albumen (13 g/L or 1.7 mM), and concentrations have been found to increase significantly with hen age (Baron et al. 2016, Jabalera et al. 2022). Ovotransferrin is a metal-binding transport protein with a high affinity for iron, binding two Fe^{3+} ions per molecule, and can also chelate multivalent ions such as zinc, copper and manganese. Due to the low concentration of iron in egg albumen (25 µM) and high concentration of ovotransferrin, all iron present in the albumen is expected to be chelated to ovotransferrin. The antibacterial (bacteriostatic) activity of ovotransferrin is due to this iron-deficient environment inhibiting bacterial growth as iron is an important cofactor for bacterial proteins. Antimicrobial activity may also arise from direct binding of ovotransferrin to the bacterial membrane. In addition, ovotransferrin may have a bacteriocidal effect via binding of divalent cations which are important for bacterial membrane integrity. One study found a correlation between increasing inhibition of *S*. Typhimurium in albumen with increasing concentrations of ovotransferrin (Jabalera et al. 2022).

To achieve statistically significant data on *Salmonella* growth/survival in albumen, most data are derived from albumen inoculated with much higher numbers of *Salmonella* than would likely be encountered during a natural contamination event. This may result in an over-estimate of growth/survival due to titration of antibacterial components by high numbers of bacteria. Conversely, because higher numbers of cells would utilise any limiting nutrients and growth factors available (for example, the growth-limiting concentrations of iron available), fewer growth generations might occur when using high compared with low inoculum numbers.

Despite the inhibitory nature of albumen, some *Salmonella* will persist and some data suggests that slow growth could occur. The temperature at which eggs are stored affects various properties of the albumen, which in turn may affect *Salmonella* survival. Experimental data on the behaviour of *Salmonella* in albumen published since 2016 has been compiled in Table 3, and earlier studies were discussed in previous Risk Profiles. Information on *Salmonella* growth/survival in albumen is often derived from studies using inoculation of isolated and homogenised albumen. Although this is a convenient system and relevant to product sold as liquid albumen, it is an imperfect model for testing growth/survival in albumen within intact eggs. For example, albumen pH influences *Salmonella* survival, yet the changes in albumen pH over time differ between the experimental isolated albumen and albumen within intact eggs (Rehault-Godbert et al. 2010). The model also does not consider any leakage of growth-promoting nutrients from the yolk that occur over time in a temperature-dependent nature.

A recent study investigated the behaviour of a cocktail of five *Salmonella* serotypes at different temperatures in unpasteurised albumen that was separated from other egg components (Table 3) (Kim et al. 2018). There was a reduction in viability over time at 5˚C and 10˚C. At 25˚C and 30˚C, there was an approximately one-log CFU/g increase in *Salmonella* observed after four days, but numbers were similar to input numbers after 20 days. Similarly, albumen did not support the growth of ten different *S*. Enteritidis strains that were inoculated at high concentrations (10^5 CFU/ml) and incubated at 25˚C; however, strains persisted over the four-day course of the experiment (Gast et al. 2018). An earlier study examined *S.* Typhimurium and *S*. Sofia numbers after inoculation at high concentrations (5 x 10^4 CFU/ml) into albumen and incubation at 15˚C, 22˚C or 37˚C for 35 days (McAuley et al. 2015). No significant differences were observed between serotypes/strains for albumen survival or growth in this study. Minimal growth in albumen occurred at 15˚C (and in some experimental replicates, a decline in viability was observed), but growth rates increased with increasing temperature (15˚C < 22˚C < 37˚C). Another recent study examined growth of *S*. Typhimurium inoculated into the albumen of whole eggs (Khan et al. 2021). No growth of *Salmonella* in the albumen was recorded after four days incubation at 5˚C. However, a 2-log increase in numbers was observed following storage at 25˚C for 28 days.

Some reports have suggested that *S*. Enteritidis is better adapted to survive in egg albumen than other *Salmonella* serotypes (Shah et al. 2012, Baron et al. 2016, Shah et al. 2017). Information is accumulating on the genetic determinants for the survival of *Salmonella*, particularly *S*. Enteritidis, in the albumen. In a study that showed significant differences in the survival of two *S*. Enteritidis strains in albumen, there were genetic variations in 38 genes involved in a wide range of functions (Wang et al. 2018). Variations in *bioC* (biotin synthesis) and *pliC* (lysozyme inhibition) genes affected albumen survival. Disruption of *S*. Enteritidis *pliC* gene renders *S*. Enteritidis sensitive to lysozyme (Callewaert et al. 2008), and the *pliC* gene has also been identified in *S*. Typhimurium (Leysen et al. 2011). Another study conducted a proteomic analysis of *S*. Enteritidis exposed to egg white (Qin et al. 2019). Upregulated proteins were involved in iron acquisition, cofactor and amino acid biosynthesis, transporter, regulation and stress responses. Down-regulated proteins were mainly involved in energy metabolism, virulence as well as motility and chemotaxis. Disruption of the stress-response gene, *ybgC*, and multidrug efflux transporter gene, *acrD*, resulted in decreased survival in egg white (Qin et al. 2019, Qin et al. 2021). The *tolC* gene, which encodes another outer membrane channel important for efflux of harmful molecules from the cell, was also upregulated in albumen, and important for the protection of *S*. Enteritidis against ovotransferrin-mediated inhibition in albumen (Raspoet et al. 2019). Other genes that were upregulated in albumen (*nhaA*, *cpxR* and *waaH* and *eco*) were proposed to be important for adaption to the alkaline pH and repair of envelope damage occurring during incubation in albumen (Huang et al. 2019). Other upregulated genes including *SEN1393* (involved in sulphate assimilation) and a gene with unknown function (*yoaE*) that was regulated by the CpxR protein, were also required for *S*. Enteritidis survival in albumen (Huang et al. 2019, Liu et al. 2021).

Migration through the albumen and penetration of the vitelline membrane has been reported for *S.* Enteritidis. Refrigeration helps to reduce this migration and the growth rate (Gast et al. 2013a). Yolk can also be released into the albumen as the vitelline membrane degrades over time, a process which is enhanced with increasing temperature (Whiting et al. 2000).

2.4.5 *Salmonella* behaviour in egg yolk and liquid whole egg

Experimental data assembled in the 2011 and 2016 Risk Profiles (Lake et al. 2011, Rivas et al. 2016) suggested that *S.* Enteritidis and *S.* Typhimurium could grow in whole egg, egg yolk or whole liquid egg at ≥7˚C but not at 4˚C. For example:

- One study measured and modelled the growth of *S*. Enteritidis in pasteurised whole liquid eggs with varied concentrations of salt, at three pH levels, and at temperatures in the ranges 1-25˚C (Jakočiūnė et al. 2014). Under the cooler temperatures, without added salt and at pH 7, the model predicted that *S*. Enteritidis could grow at temperatures above approximately 3˚C (very slowly, 0.01 divisions/hour), and viability declined at 1˚C. Increasing the pH and/or the salt concentration inhibited growth. While this suggests that growth below 7˚C is possible, it should be noted that the model was based on experiments at 1, 7, 13, 19 and 25˚C so growth was not experimentally-confirmed in the range 3-6˚C. The potential for different *Salmonella* serotypes to grow in whole liquid egg or yolk at temperatures between 4 and 7˚C requires further study.
- Another study monitored growth of *S.* Typhimurium and *S.* Sofia in unpasteurised liquid whole egg, liquid yolk, at 15, 22 and 37˚C (McAuley et al. 2015). No differences in the growth rates were observed between strains, so the researchers pooled the results. As expected, growth was significantly greater in the egg yolk and whole egg than in egg white, and the growth rate increased with higher temperatures. In egg yolk and whole egg at the same temperature, the combined growth rates were 0.842 and 0.612 $\log_{10}$ CFU/ml/h, respectively. At 15˚C, the time to reach stationary phase (10^8-10^9 CFU/ml) was three days in yolk and four days in whole egg.

Data from studies published since 2016 are shown in Table 3, and are consistent with earlier findings. One recent study investigated the growth of a cocktail of five *Salmonella* serotypes in unpasteurised, liquid egg products (yolk and liquid whole egg - albumen and yolk) at different temperatures; the results for selected conditions and timepoints are shown in Table 3 (Kim et al. 2018). For yolk and liquid whole egg, no growth occurred at 5˚C and the numbers recovered gradually declined over time. At temperatures of 10˚C to 25˚C, growth rate increased with increasing temperature, with a five-log CFU/g increase in *Salmonella* occurring after about 12 hours in yolk at 25˚C. Similarly high growth rates were observed at 35˚C and 40˚C (data not shown in Table 3). The growth rate was slightly higher at each temperature in yolks compared with liquid whole egg. The data have been used to produce a predictive model for *Salmonella* growth in liquid eggs.

Another study that inoculated *S*. Typhimurium into the egg yolk of whole eggs demonstrated significant growth to 7.88 $\log_{10}$ CFU/ml by day 4 of storage at 25 ˚C (a ~3.9 log-increase in CFU/ml); growth plateaued from day 4 until day 28 (Khan et al. 2021). At this temperature, increasing numbers of *Salmonella* were also recorded in the albumen from yolk-inoculated eggs, and conversely, increasing numbers of *Salmonella* were recorded in the yolk from albumen-inoculated eggs, thus, migration from the albumen to the yolk occurred. No growth of *Salmonella* was recorded after four days incubation at 5˚C in yolk (or albumen, as discussed in Section 2.4.4), but the numbers were significantly higher in the yolk suggesting better survival. The study also demonstrated that the various key genes involved in *Salmonella* virulence and invasion in mammalian hosts were differentially expressed at the different storage temperatures, and in yolk compared with the egg surface or albumen. There was also

a significantly higher rate of salmonellosis for mice that were fed *Salmonella*-inoculated yolk that had been incubated at 25˚C compared with 4˚C, and compared with inoculated albumen or eggshell wash. However, this might also be due to the lower *Salmonella* numbers present in the other treatments.

Like other studies, there was rapid multiplication of ten *S*. Enteritidis strains that were inoculated into separated yolk and incubated at 25˚C (Gast et al. 2018). The authors noted that although refrigeration will restrict growth, the internal egg temperature will take time to cool, which highlights the importance of prompt refrigeration of eggs.

Table 3. Behaviour of *Salmonella* in whole eggs or egg contents at egg storage-relevant temperatures (studies published since 2016).

Serotype	Experimental setup	Storage conditions	Change in numbers (log_{10} CFU/unit)[1]	Reference
Typhimurium	0.1 ml inoculum of 3 log_{10} CFU/egg directly injected into albumen or yolk of intact egg	25˚C, albumen:		(Khan et al. 2021)
		4 days	NC	
		28 days	↑2.0	
		25˚C, yolk:		
		4 days	↑3.9	
		28 days	↑~4.0	
Cocktail: Enteritidis, Gallinarum, Typhimurium monophasic, Bareilly, Richmond	Inoculum added to unpasteurised liquid egg components at 3 log_{10} CFU/g	5˚C, 10˚C albumen:		(Kim et al. 2018)
		10 days	↓~0.5	
		20 days	↓~2.0	
		40 days	ND	
		25˚C, 30˚C, albumen:		
		3 days	↑~1.0	
		10 days	NC	
		20 days	NC	
		5˚C, yolk:		
		10 days	↓~0.5	
		20 days	↓~1.0	
		10˚C, yolk:		
		4 days	↑~1.5	
		10 days	↑~4.5	
		20 days	↑~5.0	
		15˚C, yolk:		
		2 days	↑~3.0	
		4 days	↑~5.0	
		25˚C, yolk:		
		0.5 days	↑~5.0	
		1 day	↑~5.0	
Enteritidis (10 isolates tested individually; ranges reported here)	0.1 ml inoculum in saline added to separated yolk at 1 log_{10} CFU/ml or albumen at 5 log_{10} CFU/ml	25˚C, yolk:		(Gast et al. 2018)
		6 hour	↑1.3-1.8	
		24 hour	↑7.0-7.5	
		25˚C, albumen:		
		1 day	NC-↓1.2	
		4 days	NC-↓1.1	

[1] ↓ = decreased by >0.5 log_{10} relative to starting numbers; ↑ = increased by >0.5 log_{10} relative to starting numbers. Data are reported from text, if provided, or estimated from graphs. CFU, colony forming units; NC. no change (change ≤0.5 log_{10}); ND, not detected.

2.4.6 *Salmonella* behaviour during egg processing

Requirements for egg processing in New Zealand are outlined in the *Animal Products Notice: Production, Supply and Processing* (Ministry for Primary Industries 2022c), and procedures are listed in the *RMP Template for Harvesting, Candling, or Packing Eggs* (Ministry for Primary Industries 2020). The general steps involved in egg primary processing in New Zealand are shown in Figure 4. Key steps involve:

- Harvesting eggs,
- Egg washing (optional),
- Candling eggs (assessing eggs for freshness, fertility, or defects such as cracks by use of light, or other candling or defect assessment method, if validated),
- Grading eggs, and
- Packing whole shell eggs.

Eggs for sale in New Zealand must be visibly clean. Egg washing/sanitising is optional in New Zealand. Egg washing/sanitising may reduce faecal contamination and *Salmonella* numbers on the surface of eggs. However, it must be carried out in a manner that does not increase the likelihood of shell penetration, such as damaging shell integrity, creating a negative temperature gradient that might suck *Salmonella* across the eggshell into the egg contents, or creating condensation. In a 2016 survey of New Zealand 28 egg layer farms and 26 packhouses, 38.5% of packhouses performed no egg washing, 38.5% washed only dirty eggs, and 23.1% washed all non-cracked eggs (Kingsbury and Soboleva 2019b). Options for egg cleaning procedures used in New Zealand packhouses may include:

- Dry-buffing, with a clean and sanitised dry cloth, so the egg cuticle is not damaged.
- Wet-wiping, with a clean, damp cloth, potable water and approved egg washing chemicals The procedure should not leave water droplets on the egg. The RMP Template indicates that wet wiping is not recommended.
- Washing. This may involve jets of wash water and/or brushes, or a static water bath where the water is changed regularly, whereby eggs are dipped during washing, but not soaked for an extended period of time. The wash temperature must be at least 12˚C warmer than the egg temperature but must not exceed 45˚C to avoid damaging the cuticle. The water must be potable and use approved egg washing chemicals. Eggs must be dried quickly and immediately after washing in a manner that avoids condensation forming on eggs.

Various studies have examined the effects of egg washing on *Salmonella* numbers, but the benefits have been debated due to the concerns that the process may promote *Salmonella* internalisation of eggs (Whiley and Ross 2015). In a recent study, eggs laid by chickens that were experimentally infected by *S*. Typhimurium were washed by massaging in 0.5% Circhlor solution for 30 seconds, transferred to the 0.4% Virogard for 10 seconds and dried (McWhorter and Chousalkar 2020). The mean proportion of *Salmonella*-positive eggshell surfaces before washing was 0.18 ± 0.06. After washing, a significant ($p < 0.05$) reduction in the mean proportion of positive samples (0.03 ± 0.03) was observed. The effect on *Salmonella* present within egg pores was also tested by washing off any *Salmonella* present off the eggshell surface, crushing the shells, and incubating them in Buffered Peptone Water. Egg washing was not found to impact the prevalence on *Salmonella* within egg pores, with similar mean proportions for both washed (0.25 ± 0.07) and unwashed (0.23 ± 0.07) eggs.

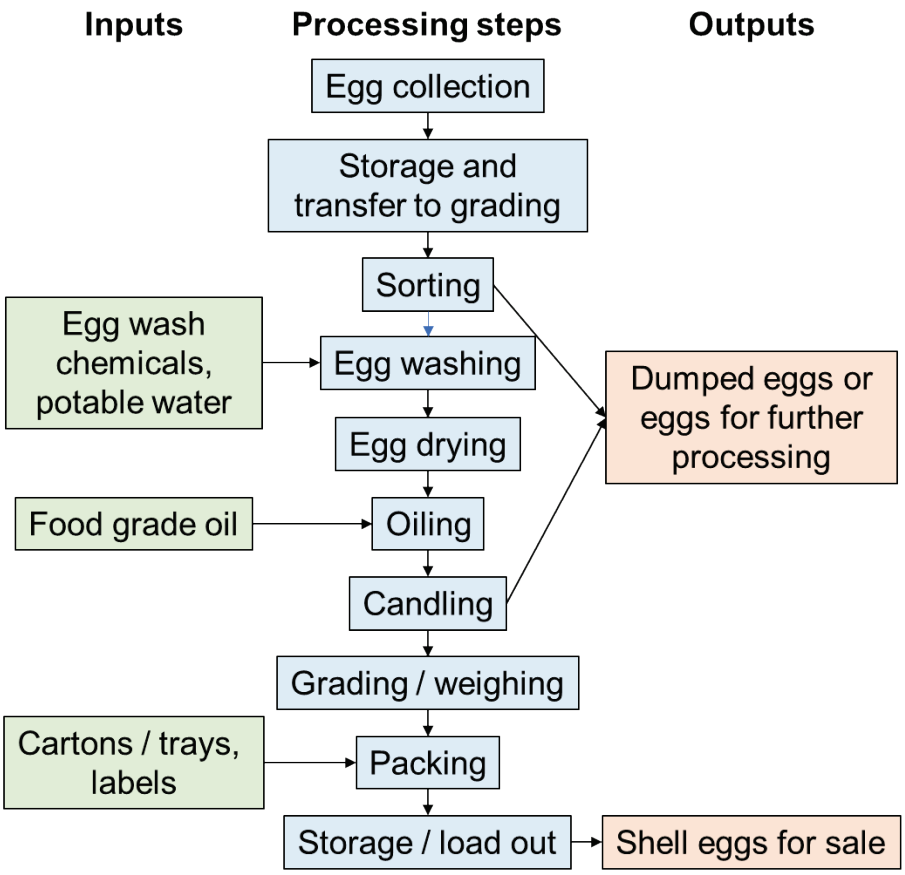

Figure 4. Generic process flow diagram for egg primary processing in New Zealand. Adapted from Ministry for Primary Industries (2020).

Sanitisers commonly used internationally in egg washes are mostly chlorine-based; for example, 100-200 ppm chlorine is used in the US (Jones et al. 2021). Other agents based on iodine, hydrogen peroxide, ozone, peracetic acid (PAA), quaternary ammonium compounds, calcium hydroxide and plasma-activated water treatment are also either in use, or have been investigated for use as antimicrobial egg washes (Al-Ajeeli et al. 2016, Alam et al. 2018, Lin et al. 2019, Grudlewska-Buda et al. 2022, Bermudez-Aguirre and Niemira 2023, Lin et al. 2023). For example, one study showed that 50–100 ppm PAA is equivalent to 100–200 ppm chlorine in reducing egg surface microorganisms, and higher concentrations of 400–500 ppm PAA resulted in a lower incidence of viable but not culturable *Salmonella* (Jones et al. 2021).

Oiling of egg surfaces may also be carried out in some New Zealand packhouses. The application of an oil coating (for example, mineral oil) has been reported to preserve the internal quality of eggs by slowing the loss of water and CO_2 (Ryu et al. 2011, Figueiredo et al. 2014, Sharaf Eddin et al. 2019). It has also been proposed to obstruct eggshell pores, thereby inhibiting *Salmonella* internalisation from shells. One study reported that there was no *Salmonella* detected in contents from oiled or non-oiled eggs, but that oiling did not influence the overall microbiological quality of the eggs (Figueiredo et al. 2014). Studies and reviews have examined the application to eggs of a range of food-safe waxes, oils, proteins and polysaccharide coatings, including those which have various antimicrobials, bacteriophages and antioxidants incorporated, to reduce the surface microbial numbers of eggs and increase

shelf life (Hong et al. 2016, Upadhyaya et al. 2016, Pires et al. 2019, Sharaf Eddin et al. 2019, Azari et al. 2023, Bermudez-Aguirre and Niemira 2023).

Ultraviolet (UV) light treatment is also implemented in some New Zealand packhouses for reducing microbial numbers on eggshells. Various light-based technologies have been explored for the surface decontamination of eggs in addition to UV, such as near UV–visible light, UV, pulsed light, high-intensity light pulses, blue light and light emitting diodes (Holck et al. 2018, Mattioli et al. 2020, Bermudez-Aguirre and Niemira 2023). Depending on the treatment and processing conditions, at least a two-log reduction of *Salmonella* numbers has been reported (using UV, 254 nm for 15 seconds); higher reductions have been obtained using pulsed light, irradiation, and high voltage cold plasma (reviewed by (Bermudez-Aguirre and Niemira 2023)). Efficacy of light-based treatments is limited by radiation transfer through opaque surfaces; as such, it would be expected to be less effective on bacteria residing in protected eggshell pores or in faeces on egg surfaces.

2.4.7 *Salmonella* behaviour during pasteurisation and cooking

Table eggs, processing grade eggs and cracked and broken eggs that are not leaking may be sent for pulping and pasteurisation (Ministry for Primary Industries 2019a). Pasteurised egg pulp products must be stored at ≤6˚C, and have a shelf life of seven days if chilled immediately, or indefinitely if frozen. These processed and convenient forms of eggs are commonly used for foodservice and in commercial kitchens. The 2016 Risk Profile (Rivas et al. 2016) included data on pasteurisation, D-times and survival during cooking for *Salmonella* in eggs and egg products. These include:

- D-values in intact, whole eggs were $D_{58˚C}$ = 4.5 minutes and $D_{57˚C}$ = 6.0 minutes;
- D-values for liquid yolk were $D_{61.1˚C}$ = 0.57 minutes and $D_{63.3˚C}$ = 0.2 minutes and this increased with added sucrose or salt;
- D-values for liquid whole egg at 60˚C ranged from 0.31-0.69 minutes.
- Liquid albumen requires pasteurisation at lower temperatures (<60˚C) to retain functionality, so D-values tend to be longer (for example, $D_{52˚C}$ ranged 3.7 to 13.4 minutes for different serotypes); and
- *Salmonella* can survive cooking processes that result in undercooked eggs (e.g. runny yolk).

New Zealand food processors that pasteurise eggs are referred to the recommended pasteurisation regimes in the *Australia New Zealand Food Standards Code Standard 4.2.5* (Primary Production and Processing Standard for Eggs and Egg Product).[14,15] Adherence to this Standard is only a regulatory requirement in Australia. The Standard specifies the following minimum temperature/times; following treatment, each product type must be immediately cooled to a maximum temperature of ≤7˚C:

- Egg pulp (egg contents, without added sugar or salt): 64˚C/2.5 minutes.
- Liquid egg yolk: 60˚C/3.5 minutes.
- Liquid egg white: 55˚C/9.5 minutes.

[14] https://www.mpi.govt.nz/food-business/poultry-egg-processing-requirements/egg-production-processing/egg-production-processing-food-safety-requirements/; accessed 10 May 2023.
[15] https://www.legislation.gov.au/Details/F2018C00937; accessed 10 May 2023.

A report from EFSA expressed a lack of certainty that the pasteurisation processes used by industry effectively eradicated *Salmonella*, and recommended validation of the current industrial processes (European Food Safety Authority Panel on Biological Hazards 2010b). In the US, *Salmonella* is occasionally isolated from pasteurised egg products by food manufacturers or the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) and may be present as a result of either the presence of pasteurisation-resistant bacteria or post-processing contamination (Gurtler et al. 2013). In addition, there have been recalls for *Salmonella* isolated from pasteurised egg product (Table 20, Appendix). The above information on D-times suggests that pasteurisation regimes recommended for New Zealand egg product manufacturers will be effective, but specific experiments investigating these conditions (and the actual conditions used in the industry) using serotypes isolated from New Zealand eggs would provide further assurance.

The USDA FSIS is responsible for regulating egg products in the US and requires liquid whole egg to be pasteurised at 140˚F (60˚C) for a minimum of 3.5 min, after which it may be served to consumers with no further interventions to inactivate bacteria (9 CFR 590.570).[16] As discussed in Appendix C.2.5, all eggs in the US that are diverted from a farm that tests positive for *S.* Enteritidis must be treated by a process such as pasteurisation that results in a 5-log reduction of *S*. Enteritidis numbers. In experiments applying the recommended pasteurisation treatment, 20 *Salmonella* strains (half Enteritidis, all non-Typhimurium) were each recoverable from liquid whole egg if inoculated at a 4.5 $\log_{10}$ CFU/ml, but not when inoculated at 3.5 $\log_{10}$ CFU/ml (i.e. the final concentration was <1 CFU/ml) (Gurtler et al. 2015). There were differences in survival between the strains, and the D-values in liquid whole egg at 60˚C ranged from 0.34 to 0.58 minutes. Furthermore, original pasteurisation time and temperature requirements for liquid egg whites were based on a pH of 9 for egg whites, while under current practices eggs typically have a pH of 7.8 when they reach processing. The reduced pH makes pasteurisation less effective (United States Department of Agriculture Food Safety and Inspection Service 2020). Taken together, the data showed that *Salmonella* could survive the recommended pasteurisation regime if present in sufficiently high numbers, and provided evidence of inter-strain survival differences.

Two other studies that evaluated the US pasteurisation time/temperature regimes evaluated *S.* Enteritidis and *S.* Oranienburg survival in salted egg products (liquid whole egg or liquid yolk; 10% salt) and found that the required pasteurisation regime for these products (63.3˚C/3.5 minutes) would not achieve the necessary 5-log reduction (Gurtler et al. 2011, Gurtler et al. 2013). A third study developed a model for inactivation of *Salmonella* in commercial liquid egg yolk, based on survival studies of three strains of *Salmonella* (three Enteritidis, one Oranienburg) shown to have higher heat tolerance (Jordan et al. 2011). Survival curves at 58, 60, 62 and 64˚C featured a lag, followed by logarithmic (first order, kinetic) inactivation. The model predicted that pasteurisation regimes for liquid egg yolk (60˚C/6.2 min or 61.1˚C/3.5 min) would reduce *Salmonella* numbers by at least 6-log. Thermal inactivation kinetics have also recently also been determined for heat-tolerant *Salmonella* strains in liquid whole egg (Gurtler et al. 2019). This food must be heated at 56˚C, 60 ˚C and 64˚C for at least:

[16] https://www.govinfo.gov/content/pkg/CFR-2022-title9-vol2/xml/CFR-2022-title9-vol2-sec590-570.xml; accessed 29 May 2023

- 33.2, 2.7, and 0.31 min, respectively, to achieve a 4-log reduction of *Salmonella* (4D);
- 39.0, 3.1, and 0.34 min, respectively, for a 5-log reduction (5D); and
- 45.0, 3.5, and 0.39 min, respectively, for a 6-log reduction (6D).

In addition to pasteurisation, the irradiation of eggs has been investigated as a potential method to eliminate *Salmonella* from egg contents. As discussed in a review by Whiley and Ross (Whiley and Ross 2015), the minimal dose required to inactivate *Salmonella* was reported to be 1.5 kGy. However, this was shown to cause changes in organoleptic properties, which included increased egg yolk odour and decreased clarity of the egg white. Additionally, the functional properties of the egg were affected, including decreased foam stability of the egg white, which would limit the functionality and desirability of the irradiated product.

2.5 EXPOSURE ASSESSMENT

Key findings

- A 2016 cross-sectional survey of 28 New Zealand egg layer farms found a lower prevalence of *Salmonella* at the farm, shed and sample level compared with similar Australian surveys. *Salmonella* was not detected on 16 of the 28 surveyed farms, and four farms had only one positive sample. *Salmonella* was detected on egg contact surfaces at the packhouses for the three farms that had the highest prevalence of *Salmonella* in shed samples. Once contaminated, these surfaces would be a source of further contamination of eggs being processed on the same surfaces. Therefore, contamination on the outside of the egg may occur. Isolates were common New Zealand serotypes; *S.* Enteritidis was not found.
- A study that surface-inoculated clean, intact eggs with New Zealand egg-associated *Salmonella* isolates and incubated them at 15˚C or 22˚C, did not detect *Salmonella* in egg contents. While external contamination of a small percentage of eggs is likely, internal contamination of clean, intact New Zealand eggs by non-Enteritidis serotypes is thought to be a rare occurrence.
- Data from the National Microbiological Database (NMD) programme showed that the prevalence of *Salmonella* in end-of-lay poultry carcasses following primary processing remains very low (less than 1% of chicken carcasses). *S.* Enteritidis has been isolated once (in 2021) from a broiler chicken carcass during NMD programme testing, and from egg layer and hatchery environments, but not from eggs.
- There have been no egg recalls for potential contamination with *Salmonella* issued in New Zealand since at least 2011.
- Industry data shows that egg consumption in New Zealand has fluctuated since 2010, reaching an estimated peak of approximately 250 eggs per person per year during 2020. Data on egg consumption from New Zealand nutrition surveys from 2002 and 2009 indicate that at that time, almost half of the population consumed egg on any given day. Most servings of eggs were cooked but consumption of raw egg was reported by some respondents. The data do not provide information on the nature of egg cooking (such as times/temperatures or egg appearance, for example, “runny”, “soft boiled”).
- Data gaps: There have been no New Zealand surveys of *Salmonella* prevalence in or on eggs at retail since 2007. The most recent national nutrition surveys on consumption data were in 2002 (for children) and 2009 (for adults) which might not reflect current egg consumption. There are also no new data on domestic handling, storage and cooking of eggs by the New Zealand consumer.

2.5.1 New Zealand prevalence studies

Testing programmes

Environmental testing of New Zealand poultry flocks for *S*. Enteritidis was implemented under the Emergency Control Scheme (ECS; Section 4.1). There were 197 operators listed as having a different RMP or Export Approved Premises (EAP) captured within MPI's results data.[17] A total of 160,681 individual results were submitted to MPI under the ECS (during the period 6 October 2021 to 5 October 2022). *S*. Enteritidis was 'confirmed' or 'detected' in 46 samples, and 168 samples were listed as Presumptive *S.* Enteritidis-positive. These were from five different RMP or EAP/*S*. Enteritidis Emergency Control Scheme operators. These results reflect a period of time during which *S*. Enteritidis was being managed through an emergency response. The data do not indicate the current situation nor the situation before October 2021.

Over the 2016-2022 period, NMD Programme testing detected *Salmonella* from 2/1,476 (0.14%) from end-of-lay (EOL) carcass rinsates samples (Table 10, Appendix). Serotypes of the two isolates included *S*. Senftenberg and *S*. Brandenburg. For 2015-2022, prevalence for broiler chicken carcass rinsates was similarly low at 8/16,899 (0.05%). Not all EOL flocks are sent for primary processing. Also, NMD programme samples are collected from carcasses at the end of processing, so results do not reflect on-farm prevalence. However, the low prevalence following primary processing is consistent with a low on-farm prevalence, and serotypes identified shed light on serotypes that are likely present on layer farms, and which might contaminate eggs.

2016 Cross-sectional survey of the New Zealand egg layer environment

The 2016 Risk Profile (Rivas et al. 2016) highlighted that there were no data on *Salmonella* prevalence in New Zealand layer flocks or layer farm environments. The report recommended a separate study to gather information on the prevalence of *Salmonella* and the potential for *Salmonella* to contaminate eggs, via environmental sampling of New Zealand layer farms. In response to the recommendations, a 2016 study surveyed the prevalence of *Salmonella* in the New Zealand commercial egg layer environment from 67 sheds on 28 of the 143 layer farms operating in New Zealand at the time (Kingsbury et al. 2019a). The 28 farms represented 20% of the total egg producers, and contained 46.0% of total laying hens (1.60 million of 3.48 million) in New Zealand. The sampled sheds encompassed all housing systems, single and multi-aged flocks, and selected farms included all production sizes (from 500 to 400,000 birds per farm). Samples for the New Zealand survey included farm-level feed, laying shed dust, fresh faeces and boot/manure belt swabs. Egg contact surfaces in egg collection and packing areas were also sampled since these are important potential sites for external contamination of eggshells (Davies and Breslin 2003, Dewaele et al. 2012b, Utrarachkij et al. 2012).

The prevalence of *Salmonella* in the New Zealand egg production environment was found to be low compared with similar Australian or international studies (Table 12, Appendix). *Salmonella* prevalence was also lower at the New Zealand layer shed (31.3%) and farm level

[17] Some of these operators have ceased commercial production. The number of sites covered by each RMP/EAP was not available, and there are some operators with multiple RMPs.

(42.9%) compared with similar New South Wales (49.6%-positive sheds, 44.9%-positive farms) and Queensland cross-sectional egg layer surveys (43.4%-positive sheds, 57.1%-positive farms) (New South Wales Food Authority 2013, Cuttell et al. 2014). The lower farm and shed prevalence from the New Zealand survey was even more striking considering that 16.4% of positive sheds and 14.3% of positive farms in this study were based on positive dust samples only, which was not directly sampled in the Australian surveys. Because dust samples may be more likely to be positive than other shed sample types, the Australian prevalence data are possibly an underestimate. While the overall prevalence of *Salmonella* on New Zealand layer farms was low relative to international surveys, some farms had a high prevalence of *Salmonella*-positive environmental samples. *Salmonella* was also detected on egg contact surfaces from the egg packhouse of three farms that had a high *Salmonella* prevalence in laying sheds. Once contaminated, these surfaces would be a source of contamination to eggs subsequently processed on them.

2.5.2 Serotypes from New Zealand poultry

The ESR Enteric Reference Laboratory (ERL) receives isolates of *Salmonella* from non-human samples. Examples of sources include the poultry environment, poultry feed, and miscellaneous sources.[18] Poultry isolates arise from a range of different programmes and mechanisms, which include but are not limited to:

- Poultry environmental isolates from on-farm testing. Previously, testing was only conducted on some farms and there was no standardisation of testing between farms. With the implementation of the ECS structured surveillance system and routine environmental sampling for *S*. Enteritidis, isolates are also being reported through this mechanism if they are sent to ESR ERL.
- Feed isolates from feed producers from poultry industry laboratories
- Poultry carcass rinsate samples (including from EOL chickens) collected via the NMD Programme (Appendix A.4.1).
- Food isolates obtained from outbreak investigations.

Overall, the system is a passive surveillance system, but isolates from both active and passive surveillance programmes, and *ad hoc* testing, feed into this stream. The rationale and requirements for sampling within each project differs; therefore, there is significant bias in the animals and environments for which data are available. As such, data do not represent true prevalence in animals or the environment. The sampling frame, coverage of population, and number of samples taken within each programme that feed into this surveillance stream differ depending on the requirements of the programme.

Common serotypes of *Salmonella* isolated from poultry sources over the period 2015 to 2022 are presented in Table 4. Of the 6,700 isolates received by ESR ERL, 816 (12.2%) were from poultry sources, the majority of which were from the poultry production environment. Higher numbers were received from poultry environmental samples in 2021 and 2022 than previous years due of testing as a consequence of the *S*. Enteritidis outbreak associated with poultry. There were 11 serotypes which were identified 10 or more times. The most commonly reported was *S*. Enteritidis (274 isolates; 33.6% of all poultry isolates). The second most common

[18] https://www.esr.cri.nz/our-research/nga-kete/infectious-disease-intelligence/enteric-reference-testing/non-human-salmonella-isolates/; accessed 18 May 2023

serotype was *S*. Typhimurium (166 isolations; 20.3% of all poultry isolates). All *S*. Enteritidis isolates were reported in 2021 and 2022; the serotype had not been isolated from New Zealand poultry prior to this although a 2020 isolate was later typed as *S*. Enteritidis during the SE_2019_C-01 outbreak investigation (Ministry for Primary Industries 2021. The increase in poultry isolate referrals in 2021 and 2022 (354 and 236 isolates respectively, compared with a range of 24 to 52 isolates over the years 2015 to 2020) is in large part due to the increased environmental testing following the discovery of the *S.* Enteritidis incursion in poultry flocks (Section 4.1). Because the focus of testing was for detecting *S*. Enteritidis, other serotypes isolated may not have been sent to ESR for additional typing, thus data do not reflect the true proportions of serotypes present. The next four most common serotypes reported were *S*. Mbandaka (66 isolations; 8.7%), *S*. Give (46 isolations; 6.1%), *S*. Thompson (45 isolations; 6.0%) and *S*. Infantis (34 isolations; 4.5%). The majority of *S*. Give isolations were from 2022 and an increase was noted in the number of this serotype from non-clinical sources for this year.[19]

A further data source for the prevalence of *Salmonella* in poultry is annual data from the poultry industry, which is reported in the *Surveillance* biosecurity magazine published by MPI.[20] The data are received from poultry-testing laboratories and include poultry feed testing, broiler samples (including from the NMD programme), and environmental samples. There is some overlap between this data stream and the ESR ERL reporting; for example, both include data from the NMD programme. However, many isolates serotyped by poultry laboratories were not sent to ESR ERL for further typing, and thus not included in the ESR ERL surveillance reporting. From the period 2015 to 2021, there were 1,193 *Salmonella*-positive samples from 76,836 tested (1.6%) (Table 11, Appendix). The most common serotype isolated was *S*. Mbandaka (156 detections from 1,193 isolates; 13.1%), followed by *S*. Bovismorbificans (144/1,193 detections; 12.1%) and *S*. Enteritidis (123/1,193; 10.3%). The serotype proportions differed between ESR ERL poultry data and poultry industry data; for example, while *S*. Typhimurium was the second most commonly identified serotype in ESR ERL data, it was the sixth most common from the poultry industry data.

The serotypes identified from the New Zealand egg layer farm survey included *S*. Infantis, *S*. Thompson, *S*. Typhimurium, *S*. Mbandaka and *S*. Anatum (Kingsbury et al. 2019a). All of these are commonly isolated from the environment in New Zealand (Table 4, Table 11), and are amongst the most common *Salmonella* serotypes identified on egg layer farms world-wide. *S*. Enteritidis was not identified in this survey.

[19] https://www.esr.cri.nz/our-research/nga-kete/infectious-disease-intelligence/enteric-reference-testing/non-human-salmonella-isolates/; accessed 18 May 2023
[20] https://www.mpi.govt.nz/biosecurity/about-biosecurity-in-new-zealand/surveillance-biosecurity-magazine/; accessed 2 March 2023

Table 4. *Salmonella* serotypes identified 10 or more times from isolates submitted to the Enteric Reference Laboratory from poultry environmental (E), feed (F) and miscellaneous including product (M) sources (2015-2022).[1]

Serotype	2015			2016			2017			2018			2019			2020			2021			2022[2]			Total
	E	F	M	E	F	M	E	F	M	E	F	M	E	F	M	E	F	M	E	F	M	E	F	M	
Enteritidis																			172		5	62[2]		35[2]	***274***
Typhimurium	14	3		9	2	2	9	1	1	12	3	7	11		3	12	1	3	37	2	7	12		15	***166***
Mbandaka	2						1						5	3	1	4		1	27	6	1	10		5	***66***
Give																2			7	1	7			29	***46***
Thompson													1						43			1			***45***
Infantis	2				1		2					1	1	1		1			14			11			***34***
Senftenberg	2	2					1	1		1	2	1	3	2		1			2			2	1	8	***29***
Agona	4			1				1		1			2	1			1		1		3	2		5	***22***
Bovismorbificans	1			1			3			5			1						2			2			***15***
Emek											1							1	6					3	***11***
Anatum		3						1		2	1			1	1								1		***10***
Total poultry isolates	***46***			***24***			***27***			***45***			***52***			***32***			***354***			***236***			***816***
Total typed	***637***			***684***			***972***			***848***			***926***			***833***			***1,015***			***785***			***6,700***

[1] Source: https://surv.esr.cri.nz/enteric_reference/nonhuman_salmonella.php, accessed 27 June 2023.
[2] Numbers include *S.* Enteritidis isolates that were received by ESR but were typed by other laboratories.
[3] Higher numbers of isolates were received from poultry environmental samples in 2021 and 2022 than previous years due of increased testing as a consequence of the *S.* Enteritidis outbreak associated with poultry. *S.* Enteritidis is the only serotype which is required to be reported.

2.5.3 Product surveys

There have been no recent retail surveys investigating the presence of *Salmonella* in and on eggs in New Zealand. The last survey was undertaken in 2007 where *Salmonella* was isolated from nine shell surface samples (1.8% of pooled samples, each containing six eggs). There were <5 MPN/egg on eight externally contaminated eggs and 44 MPN/egg on a ninth contaminated egg. All positive samples were from cage laid eggs, and all isolates were identified as *S*. Infantis. No egg contents (3,710 eggs) were positive for *Salmonella*. Of the egg samples that tested positive for *Salmonella*, 4/9 sample units contained "dirty" eggs (obvious contamination of shell with faecal, feather or other organic material).

2.5.4 Product recalls

Between January 2015 and January 2023, there have been no New Zealand recalls issued for eggs or egg products due to *Salmonella* (as assessed from the MPI recalled food products list).[21] There were also no recalls identified between 2011 to 2015 in the 2016 Risk Profile (Rivas et al. 2016).

Recalls may be initiated following traceback investigation from cases when there was a strong association with illness and the consumption of a particular food. However, a suspect batch of eggs will likely have been consumed by the time there is a positive salmonellosis diagnosis, and particularly following later WGS to link multiple cases to a common source.

Even during the *S*. Enteritidis DT8, ST11 outbreak associated with broiler meat and eggs, no egg batches were confirmed as a source of the outbreak strain. Instead of recalls, there were increased communications and media releases issued by MPI regarding the potential for the strain to be contaminating eggs, and how consumers should manage the risk once eggs were purchased.[22]

2.5.5 Egg consumption

Eggs are commonly consumed in New Zealand. Based on egg production data, as at 30 June 2021, egg consumption in New Zealand was approximately 237 eggs per person for the year.[23] Estimated consumption amounts have fluctuated since 2010, reaching a peak of approximately 250 eggs per person per year during 2020 (Figure 5).

[21] https://www.mpi.govt.nz/food-safety-home/food-recalls-and-complaints/recalled-food-products/; accessed 6 February 2023
[22] 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/; accessed 19 June 2023
[23] https://www.eggfarmers.org.nz/egg-farming-in-nz; accessed 6 February 2023

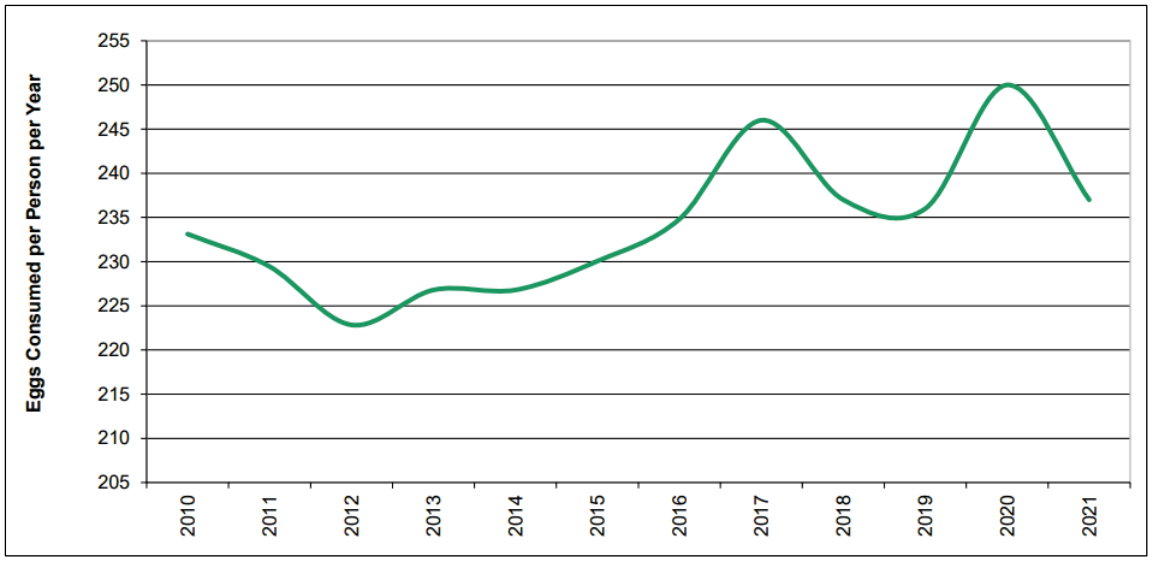

Figure 5. Estimated New Zealand annual egg consumption per capita (2010 to 2021). Graph reproduced from https://www.eggfarmers.org.nz/.

Additional data for New Zealand egg consumption were captured in the 2016 Risk Profile (Rivas et al. 2016) and based on data obtained from 24-hour dietary recall (24HDR) records collected as part of the 2002 National Children's Nutrition Survey (2002CNS, children 5-14 years, *n* = 3275; (Ministry of Health 2003)) and the 2009 Adult Nutrition Survey (2009ANS, adults ≥15 years, *n* = 4721 (University of Otago and Ministry of Health 2011)). These data are summarised in Table 5.

Further analysis of the datasets revealed that eggs were commonly consumed by both adults and children, although children consumed smaller amounts in each serving. Approximately two-thirds of egg servings were for eggs as an ingredient of a recipe (for example, quiche, burgers, sandwich filling or a component of meat coatings). For eggs eaten as eggs, 32% of the respondents reported consuming such dishes in the previous 24 hours. The most common consumption forms were:

- Eggs, pan-fried/stir-fried: 36%
- Eggs, boiled: 24%
- Eggs, scrambled/omelette: 17%
- Eggs, whole, poached: 13%

A small number of servings (15 out of 1087, 1.4%) involved consumption of raw eggs. This was double the proportion reported in the 2011 Risk Profile (Lake et al. 2011) from a 1997 National Nutrition Survey for adults (1997NNS; 0.7%, 7/1031), but this finding may be an artefact of different survey approaches. The 2009ANS 24-hour dietary recall records include 10 records involving consumption of homemade mayonnaise, of which two are reported as containing eggs (most likely raw).

As discussed in Section 3.1, older populations are among those that are at higher risk for salmonellosis, and salmonellosis during pregnancy slightly elevates risks to the developing foetus. Further analyses of the data from the 2009ANS found no significant difference in the prevalence of egg consumption between adults 65 years and over and those less than 65

years (Cressey, 2013). Similarly, the egg consumption patterns of pregnant woman were very similar to those of the general population.

Note that, while these were the most recent surveys undertaken to capture consumption data, shifts in consumer food preferences, consumption amounts, and the emergence of new foods are likely to have occurred in the 14 years (for 2009ANS) or 21 years (for 2002CNS) since these surveys were undertaken. There has been a shift in consumer eating habits with increasing demand for raw and unprocessed or lightly cooked, and ready-to-eat (RTE) foods (Broglia and Kapel 2011, Kretser et al. 2014, Whiley and Ross 2015). The increasing popularity of unprocessed home-made foods containing raw eggs such as mayonnaise, certain sauces and raw egg-based deserts like ice cream and tiramisu, and drinks such as eggnog and raw egg high protein smoothies, potentially increases the risk of salmonellosis (OzFoodNet Working Group 2015, Whiley and Ross 2015, OzFoodNet Working Group 2022).

Table 5. Consumption of eggs by adult (15+ years) and child (5-14 years) New Zealanders (national nutrition surveys).[1]

Statistic	Child (2002CNS)	Adult (2009ANS)
Number of respondents	3275	4721
Percent consumers (%)	43.8	49.7
Serving per day (consumers)	1.4	1.5
Consumer mean (g/person/day)	34.9	47.0
Population mean (g/person/day)	15.3	23.4
Serving size, mean (g)	24.6	32.3
Serving size, median (g)	9.8	11.1
Serving size, 95th percentile (g)	93.1	114.0

[1] Data extracted from (Cressey et al. 2006, Cressey 2013).

Global egg production has steadily increased, reaching 93 million tonnes in 2021, which was a 68% increase in production since 2000 (Food and Agriculture Organization of the United Nations 2022). Hen eggs accounted for 92–93% of the global egg production since 2000. In terms of kg per capita consumption for the year 2017, the European region continues to have the highest level of egg consumption (10.6 kg/capita per year) while the African region has the lowest (1.7 kg/capita per year) (Henchion et al. 2021). There has been a general upward trend in consumption since 2000, although data from the Eastern Mediterranean Region and African region were more stable over time.

2.5.6 *Salmonella* growth and control at in eggs at retail and during domestic handling

As discussed in Section 2.4, survival and growth of *Salmonella* in and on eggs depends on temperature. There are currently no data on the times and temperatures eggs are exposed to from the point of lay to the point of consumption in New Zealand. The current New Zealand requirements are that eggs carry a best before date of 35 days regardless of storage temperature (Section 4.1).

The available data indicate that survival on the shells of whole eggs varies between *Salmonella* serotypes but refrigeration temperatures improve survival (Section 2.4). Therefore, it is likely that, if present, at least some *Salmonella* can survive on the egg from the point of lay to the point of consumption. The detection of *Salmonella* from egg contact surfaces at New

Zealand packhouses and from eggs sampled at retail in New Zealand, indicates that external contamination of eggs can occur under New Zealand egg production conditions, and persist through to retail (Wilson 2007, Kingsbury et al. 2019a).

It has been shown that various *Salmonella* serotypes can penetrate the shells of eggs. However, as discussed in Section 2.4, a study that surface-inoculated clean, intact eggs with *Salmonella* egg-associated isolates and incubated eggs at 15˚C or 22˚C, did not detect *Salmonella* in egg contents (Kingsbury et al. 2019b). The 2007 survey of New Zealand eggs also did not detect *Salmonella* in egg contents (Wilson 2007). Therefore, while external contamination of a small percentage of eggs is likely, available evidence shows that the internal contamination of clean, intact New Zealand eggs by non-Enteritidis serotypes is a rare occurrence. If internal contamination were to occur, some serotypes appear to survive poorly in the albumen, and growth of all serotypes is limited. Nevertheless, the albumen is not an effective control point. If an invading *Salmonella* bacterium manages to migrate to the yolk or the yolk membrane breaks down, it could multiply in the egg contents at temperatures ≥7˚C. Similarly, *Salmonella* can grow in whole, liquid eggs (pasteurised or unpasteurised). The rate of growth is increased with increasing storage temperature. Whole, liquid eggs are likely to be refrigerated.

As discussed in the 2016 Risk Profile (Rivas et al. 2016), there is no recent information to indicate the proportions of whole eggs that are refrigerated or stored at room temperature in New Zealanders' homes, nor how long after the best before date people continue to use the eggs. Three surveys in the 1990s indicated that eggs are refrigerated in the majority of New Zealand households (56-76%). A survey of domestic refrigerators in New Zealand found one third (43/127; 34%) to be operating at a mean temperature above 6˚C (Gilbert et al. 2007). An Australian study reported that 91% of participants in a study stored eggs in the refrigerator (Whiley et al. 2017). Note that like New Zealand, there is no requirement to refrigerate eggs in Australia, but it is recommended (Section C.2).

Data from the US identified a number of examples where contradictory information was provided on the internet regarding using eggs beyond this date (Cardoso et al. 2021). Indeed, 44% of US consumers reported that they finish their egg carton regardless of the age of the eggs, and of those that discard eggs, 17% rely on smell or egg appearance. Another US survey of 1,504 adult grocery shoppers found that most (99%) stored eggs in the refrigerator for no more than 3-5 weeks (Kosa et al. 2015).

In domestic kitchens, cross-contamination of foodborne pathogens from raw eggs to hands, kitchen utensils and surfaces and devices (for example, mobile phones) can occur. Growth of *Salmonella* on contaminated surfaces will depend on the surface type, the level of contamination and organic matter, and time between contamination and cleaning. The extent of cross-contamination depends on whether mitigation practices are carried out effectively by consumers, such as cleaning contact surfaces and washing hands and equipment after handling eggs and before food preparation. In an Australian study of egg handling behaviours, only 39% of participants always washed their hands after handling eggs during food preparation, and 34% wiped down the bench after contact with raw eggs (Whiley et al. 2017). A US survey of food handling behaviours of consumers found that only 15% and 14% of consumers safely washed their hands (defined as washing hands with soap for a minimum of 20 seconds immediately after touching the raw egg and without touching anything else) after

handling raw eggs for fried eggs and for scrambled eggs, respectively (Maughan et al. 2016). Another US survey found that 48% of respondents washed their hands with soap after cracking eggs (Kosa et al. 2015). A Portuguese survey reported that 27% of consumers declared that they were unlikely to wash their hands after handling eggs during food preparation (Junqueira et al. 2022). In further surveys reviewed by Cardoso et al. (2021), 30-50% of consumers claimed to wash their hands after cracking eggs; percentages differed between consumer groups and cultures (Cardoso et al. 2021). A Canadian case–control study identified that those that do not wash hands after handling raw eggs are almost three times more likely to get infected with *S*. Enteritidis (note that *S*. Enteritidis is endemic in Canadian poultry flocks) (Middleton et al. 2014).

Pasteurisation or cooking will inactivate *Salmonella*, but the extent of inactivation depends on the temperature and time of cooking and the initial numbers of *Salmonella*. A US study found that a thermometer was never used to determine doneness for fried or scrambled eggs, and only 77% of scrambled and 49% of fried eggs reached a safe temperature (71˚C) (Maughan et al. 2016). Another US survey reported that more than half of respondents who fry and/or poach eggs cooked them so that the whites and/or the yolks were still soft or runny, a potentially unsafe practice (Kosa et al. 2015). The degree of cooking of egg depends on the culinary purpose of the egg; for example, 44% of Finnish consumers preferred soft-boiled eggs, while in Portugal, these are more commonly hard-boiled for use in salads and soups (Junqueira et al. 2022). For US consumers, 46% preferred eggs fried until the yolk is firm, while in Portugal, fried eggs are usually cooked so that the yolk remains runny and are often used as a dip for bread or fries. There are no equivalent data for New Zealand.

An Australian study identified that consumers underestimate “risky behaviours” with respect to the consumption of raw eggs (Whiley et al. 2017). Although 84% of participants indicated that they did not consume raw eggs, 86% indicated that they had eaten cake mixture/batter containing raw eggs.

2.6 DATA ON *SALMONELLA* IN AND ON EGGS FROM OTHER COUNTRIES

The 2011 and 2016 Risk Profiles (Lake et al. 2011, Rivas et al. 2016) listed data from a large number of egg surveys from many different countries. There were very few instances where the prevalence of *Salmonella* on the outside or inside of the egg exceeded 1%. Note however that the prevalence of *Salmonella* on New Zealand retail eggs from the 2007 survey was slightly higher than this at 1.8%, although small numbers of positive samples can generate large uncertainty intervals (Wilson 2007). *Salmonella* were more likely to be detected on the outside of the egg or when the whole egg (shell and contents) were analysed together. Data published since 2016 on the prevalence of *Salmonella* in or on eggs, sourced from retail of layer farms from various other countries, is provided in Table 13 (Appendix), and additional detail is included in Appendix A.5.

Recent surveys on the prevalence of *Salmonella* on Australian eggs have been published. While one study did not detect *Salmonella* on eggs at retail (0% prevalence (Symes et al. 2016)), another reported a high prevalence (5%) of *Salmonella* on eggshells (Sodagari et al. 2019). Although internal contamination of eggs has been reported in other countries, Australian studies assessed in the earlier Risk Profiles did not detect *Salmonella* in the contents of eggs at either the farm or at retail. *Salmonella* has since been detected from egg

contents sampled directly from Australian egg farms, and from eggs at retail (Crabb et al. 2019b, Sodagari et al. 2019).

There was a wide variability in *Salmonella* prevalence from egg surveys from other countries, which in part would reflect different methodologies; for example, the number of eggs included in each sample, and whether they were collected from retail or the layer shed. Prevalence on egg surfaces/eggshells ranged from 0 to 17%. Prevalence from egg contents ranged from 0 to 12%. Even within flocks that are colonised with *S*. Enteritidis that is capable of transovarian contamination of eggs, eggshell contamination has been estimated to occur at a much higher rate than contamination of egg contents (Arnold et al. 2014).

The total number of *Salmonella* present on eggs at the time of contamination will affect the likelihood and extent of both cross-contamination and trans-shell penetration. Numbers present are influenced by the source of contamination, egg handling practices on and off the farm, and time and storage conditions since contamination (Chousalkar et al. 2018a). Some data are available on *Salmonella* numbers on eggs, for example:

- Natural contamination was reported to rarely exceed 10^2 CFU/eggshell (Humphrey, 1994).
- The level of *Salmonella* on positive eggshells from an Australian free range farm were 1.7±0.1 MPN per egg (Gole et al., 2017). Similarly, there was less than 1 CFU/ml of *Salmonella* eggshell rinse, shell and membrane, and egg contents in another survey from Australian farms (Crabb et al. 2019b).

A wide variety of serotypes have been isolated from egg surfaces. Furthermore, serotypes in addition to *S*. Enteritidis have been isolated from egg contents in other international studies. These include other serotypes found to be present on New Zealand layer farms, such as *S*. Infantis, *S*. Typhimurium and *S*. Mbandaka (Crabb et al. 2019b, Sodagari et al. 2019). In Australia, a large proportion of isolates from layer poultry isolates, eggs and outbreaks associated with eggs are *S*. Typhimurium.

3 EVALUATION OF ADVERSE HEALTH EFFECTS

3.1 DISEASE CHARACTERISTICS

Key findings

- Salmonellosis is a self-limiting infection for most people. However, it can result in severe outcomes (including death) or long-term chronic conditions, particularly for the young, elderly, immunocompromised and those with underlying disease.

Information was obtained from the *Non-typhoidal Salmonellae* datasheet.[24]

Incubation: 6-72 hours, commonly 12-36 hours.

Condition: Salmonellosis, or more generally gastroenteritis or enterocolitis.

Symptoms: Self-limiting watery diarrhoea, abdominal cramping, vomiting, nausea, fever and headache. Symptoms typically last between 2-7 days.

Long Term Effects: Bacteraemia and focal systemic infections can result in up to 5% of cases. Major risk factors for invasive disease are co-infection with HIV, malaria and malnutrition. Reactive arthritis and Reiter's syndrome may develop in a small percentage of patients 3-4 weeks after enteritis. Excretion of *Salmonella* can occur for up to seven weeks after infection.

Toxins: Toxins are not produced in foods.

At risk groups: Anyone can be infected, but the young, elderly, immunocompromised and those with underlying disease are particularly at risk. The highest incidence is reported for infants <1 year and children aged 1-4 years. Although pregnant women are not thought to be at higher risk for salmonellosis, transmission of *Salmonella* to the placenta may occur on rare occasions (Coughlin et al. 2003, Tam et al. 2010). Risk factors for salmonellosis include consumption of food at retail premises, travelling abroad and contact with farm animals.

Treatment: The infection is usually self-limiting and treatment is rarely required. Uncomplicated gastroenteritis may require supportive therapy such as fluid and electrolyte replacement, especially in the elderly or young children. However, when necessary, fluoroquinolones are the antibiotic of choice. Azithromycin is a relatively new antibiotic used for multi-drug-resistant isolates.

3.2 DOSE-RESPONSE

Key findings

- There is no known safe level of exposure to *Salmonella*.
- A recent assessment found that infectivity depended on the *Salmonella* serotype. *S.* Enteritidis was three-to-four times more infectious than *S.* Typhimurium and more

[24] https://www.mpi.govt.nz/dmsdocument/1214-Non-Typhoid-Salmonellae; accessed 9 December 2022

pathogenic at low numbers, although there was a greater heterogeneity in infectivity and pathogenicity of *S*. Typhimurium.
- Dose-response is also influenced by the food type. Contamination of foods with a high fat content can increase infectivity. Foods from outbreaks associated with eggs often are high in fat content. Growth in egg yolk upregulated *Salmonella* virulence genes and resulted in a faster onset of disease and a lower infectious dose in mice.

As discussed in the 2016 Risk Profile (Rivas et al. 2016), the ability of *Salmonella* to cause illness, as reflected in its dose-response, depends on the serotype, host susceptibilities, the food matrix and the dose. The dose-response is the relationship between the number of microorganisms ingested and the probability of a specific outcome such as infection, illness or death (Bollaerts et al. 2008). Ascertaining dose-response is very challenging as it relies on data from reported outbreaks where both the human health outcomes and number of pathogenic microorganisms ingested were known, human trials (which are ethically difficult and usually involve healthy humans and not vulnerable host populations) and/or extrapolation from animal trials. The dose-response data for *Salmonella* currently rely on outbreak data and human trials. Modelling approaches attempt to account for known sources of error and variability.

A study assessed *Salmonella* dose-response using data from 35 salmonellosis outbreaks, three sporadic cases for which there was good dose information and two human volunteer feeding studies (Teunis et al. 2010). The study estimated that the number of cells that need to be ingested to cause a 50% probability of illness was as low as 36.3, although the 95% percentiles were wide (0.69-1.26 x 10^7). However, there were a number of shortcomings in this study; for example, how the unknown susceptibility status of the hosts were handled.

A more recent dose-response assessment attempted to address the limitations present in the Teunis et al. (2010) study. The study combined data from six human studies and 44 outbreaks to determine the infectivity and pathogenicity of several *Salmonella* serotypes (Teunis 2022). The study was not restricted to particular serotypes of *Salmonella*, but had a stronger focus on the two most common causing disease; *S*. Typhimurium and *S*. Enteritidis. The models estimated that *S*. Enteritidis was three to four times more infectious than *S.* Typhimurium, and three to four times more pathogenic, at low doses. However, there was more variation in pathogenicity for *S*. Enteritidis than *S*. Typhimurium.

“Infection” refers to the presence of elevated numbers of reproducing pathogens in the intestinal tract, which does not necessarily result in illness symptoms. Specifically, the model from Teunis (2022) estimated that the median dose required for 50% probability of infection by *S*. Enteritidis was very low at 1.82 x 10^0 cells (95% range of 7.25 x 10^{-1} to 3.45 x 10^2). This value was 1.78 x 10^1 cells for *S*. Typhimurium (95% range of 9.07 x 10^{-1} to 5.85 x 10^2). Data for infectivity of 11 other serotypes was more limited, which resulted in a wider range in instance in estimate uncertainty. The dose required for 50% probability of infection ranged from 2.16 x 10^0 cells for *S*. Heidelberg (95% range of 6.93 x 10^{-1} to 1.45 x 10^2), to 6.53 x 10^3 cells for *S*. Derby (95% range of 1.31 x 10^0 to 8.05 x 10^9).

“Illness” refers to when intestinal microorganisms engage in damaging activities resulting in illness symptoms, and the dose required to cause illness is often higher than the dose required to cause infection. Pathogenicity is defined as the potential for causing illness in a host. The median dose required for a 1% probability of illness was 9.89 x 10^0 cells for *S*. Typhimurium

(95% range of 3.23 x 10^{-1} to 5.72 x 10^{1}) and 6.14 x 10^{-1} cells for *S*. Enteritidis (95% range of 2.43 x 10^{-1} to 1.94 x 10^{0}). The median dose required for 50% probability of illness was 1.50 x 10^{3} cells for *S*. Typhimurium (95% range of 3.81 x 10^{1} to 8.81 x 10^{7}) and 3.36 x 10^{3} cells for *S*. Enteritidis (95% range of 1.82 x 10^{1} to 3.18 x 10^{9}).

The probability of infection also depends on other factors such as food type; for example, *Salmonella* in foods with a high fat content, and foods from outbreaks associated with eggs high in fat content appear to be more likely to cause infection (Teunis 2022).[25] Teunis (2022) compiled data from various studies on the number of people that developed illness following consumption of different egg products together with mean doses *Salmonella* for each food vehicle. For example, out of 363 exposed people, 198 developed symptoms following the consumption of eggs that had a mean dose of 10.9 CFU of *S.* Enteritidis. Studies have reported that growth in egg yolk upregulated the expression of *S.* Enteritidis virulence genes (Khan et al. 2021, Xu et al. 2022). There was also a higher probability of infection and faster disease onset for mice fed *S*. Enteritidis that had been grown in egg yolk compared with bacterial growth medium (Xu et al. 2022). These data suggest that the dose required to cause illness could be lower in egg products compared with other food types.

3.3 NEW ZEALAND HUMAN HEALTH SURVEILLANCE

Key findings

- The yearly incidence of salmonellosis in New Zealand was lower for the period covered in this report (2015 to 2021) compared with the period (2005 to 2014) covered in the 2016 Risk Profile. Lower notifications during 2020 and 2021 could be attributed to the impact of the COVID-19 pandemic public health response.
- Hospitalisation rates varied yearly from 16.4% to 30.4% of all salmonellosis cases and were the highest in 2020 and 2021. The number of hospital admissions were not higher in these years, but the numbers of hospitalisations did not show the same COVID-19 response-specific reduction as the number of notifications, which affected the rates.
- One death associated with salmonellosis occurred during the reporting period (in 2017).
- *S*. Typhimurium was the most frequently isolated serotype from human salmonellosis cases in New Zealand, followed by *S.* Enteritidis (38.2% and 12.0%, respectively, for the period 2015-2022).
- Antimicrobial resistance among non-typhoidal *Salmonella* isolated from human, animal and environmental samples in New Zealand remains relatively low compared with other countries. Rates were similar to those reported in 2014.
- Chicken eggs have been implicated as the vehicle of infection for salmonellosis outbreaks in New Zealand. For the period 2015-2021, there were six salmonellosis outbreaks where eggs were suspected or implicated with strong evidence, including 79 confirmed and 24 probable cases. These represented 13% of the total foodborne salmonellosis outbreaks and 18% of confirmed cases from foodborne outbreaks during this period. There was a single outbreak where there was strong evidence for eggs as a vehicle, although poultry meat was also considered a potential source. This comprised the 2021 *S*. Enteritidis DT8, ST11 outbreak. As of 30 May 2023, this outbreak has included 128 confirmed outbreak cases (person notified in NZ with SE genomic cluster profile Enteritidis_2019_C_01) and 6 additional epidemiologically linked cases (134 total cases). Of the 134 cases, 37% of cases were hospitalised, which was a higher percentage than for all salmonellosis cases (27%) or total *S*. Enteritidis cases (28%) over a similar reporting period.

[25] https://www.mpi.govt.nz/dmsdocument/1214-Non-Typhoid-Salmonellae; accessed 9 December 2022

- Data gaps: There have been no case control studies or source attribution studies concerning *Salmonella* and eggs in New Zealand for the period covered in this Risk Profile.

Salmonellosis is a notifiable disease in New Zealand. There are regional differences in laboratory testing methods which were originally specific to District Health Boards (DHBs), and now, to health regions under Te Whatu Ora – Health New Zealand following the dissolution of DHBs in July 2022. Diagnostic laboratories have been gradually replacing traditional culture-based methods for enteric bacteria such as *Salmonella* with culture-independent diagnostic tests (CIDT). In 2021, all community laboratories in all former DHBs except for Canterbury, South Canterbury, and West Coast had implemented screening of faecal specimens for enteric bacteria using multiplex PCR-based assays. From 2015 onward, nationally reported notification rates are a mixture of notifications based on PCR and non-PCR approaches. Multiple different testing related factors (for example, change in sensitivity due to different methods used, proportion of faecal specimens being tested) may affect the notification rates for some pathogens. However, initial analyses comparing notification trends for bacterial infections in areas using PCR-based testing and areas yet to change to CIDT suggest the change in methodology is not causing a significant increase in reported rates of salmonellosis.

Diagnostic laboratories in New Zealand routinely submit all *Salmonella* isolates to the ESR ERL for further typing (discussed in more detail in Appendix A.1). All isolates are serotyped and a subset undergo antimicrobial susceptibility testing. Prior to 1 November 2019, ESR conducted phage typing for the Typhimurium and Enteritidis serotypes (as well as *S*. Typhi). After this time, phage typing was replaced with whole genome sequencing for *S*. Typhimurium and *S*. Enteritidis, which returns a Achtman 7-gene ST (Achtman et al. 2012). Pulsed-Field Gel Electrophoresis (PFGE), which was previously considered the 'gold standard' for the subtyping of *Salmonella* (Wattiau et al. 2011, Besser 2015, Neoh et al. 2019), was used by ESR for salmonellosis outbreak investigations until November 2019 (see Appendix A.1). This was then replaced by WGS-based cluster comparisons of isolates at the SNP difference level. Compared with PFGE, this approach is not subject to interpretation error, provides a substantially higher fine typing discriminatory power for surveillance and outbreak investigations, and facilitates the improved detection of smaller and geographically widespread clusters (Chattaway et al. 2019).

3.3.1 Salmonellosis in New Zealand

The annual rates of non-typhoidal salmonellosis have slowly decreased in New Zealand since 2007, although the rates in recent years have been more static (Figure 6 and Figure 7). The 2016 Risk Profile (Rivas et al. 2016) showed that the annual rate of salmonellosis between the years 2005 and 2014 was the highest in 2005 (33.7 cases per 100,000 population) and lowest for 2014 (21.2 cases per 100,000 population). The yearly incidence of salmonellosis notifications from 2015 and 2019 ranged between 22.5 and 24.2 cases per 100,000 population in 2018 and 2019, respectively (Table 6).

Notifications were much lower in 2020 (708 cases; 13.9 cases per 100,000 population) and 2021 (714 cases; 13.9 cases per 100,000 population), which could be attributed to the impact of the COVID-19 pandemic public health response. Public health and social measures to

prevent the spread of COVID-19 in New Zealand were introduced in March 2020 and remained in place throughout 2021, with restrictions ending on 13 September 2022.[26] However, the degree of stringency of measures differed over this time period. The multiple aspects of the response listed below make it difficult to attribute any changes to notification rates to specific COVID-19 related factors or to true changes in disease incidence.

- Changes in testing priorities of laboratories, with resources diverted to the COVID-19 response.
- More emphasis on personal hygiene; for example, hand sanitiser use.
- Travel restrictions within New Zealand and overseas.
- Physical distancing requirements and limits on hospitality businesses leading to less socialising and private functions.
- Changes in the food supply; supermarkets, corner stores/dairies and convenience stores were the main food retailers open during lockdown periods; restaurants, cafes and takeaway shops were closed or had limited functionality depending on the level of lockdown and often were modified to be contactless, possibly resulting in more home cooking and more takeaway food consumption..
- Behavioural changes such as fewer visits to healthcare providers.

Table 6. Notification rates for salmonellosis as a primary or secondary diagnosis in New Zealand from 2015 to 2021.

Year	Number of cases	Incidence (cases/100,000)	Hospitalisation of cases (% of notifications)[1]	Number of cases who died (% of notifications)	References
2015	1051	22.9	172 (16.4)	0/1051 (0)	(Lopez et al. 2016)
2016	1091	23.2	207 (19.0)	0/1091 (0)	(Pattis et al. 2017)
2017	1119	23.3	214 (19.1)	1/1119 (0.1)	(Pattis et al. 2019b)
2018	1100	22.5	227 (20.6)	0/1100 (0)	(Pattis et al. 2019a)
2019	1188	24.2	230 (19.4)	0/1188 (0)	(Pattis et al. 2020)
2020[2]	708	13.9	165 (23.3)	0/708 (0)	(Horn et al. 2021)
2021[2]	714	13.9	217 (30.4)	0/714 (0)	(Pattis et al. 2022)

[1] Cases hospitalised may not be notified on EpiSurv. Therefore, percentages are indicative only since hospitalisation and notification data sources differ. See references for details of data sources.

[2] The lower-case numbers can be attributed to the impact of the COVID-19 public health response.

In the 2016 Risk Profile (Rivas et al. 2016), hospitalisation rates for the years 2005 to 2014 ranged from 12.5% to 19.2%. Hospitalisation and fatality rates for notified cases of salmonellosis in New Zealand are shown for the years 2015 to 2021 in Table 6, and for 2007 to 2021 in Figure 7. These outcomes are not always reported for each case, so percentages expressed in terms of the total number of case notifications may differ slightly from the true percentages. The number of hospital admissions with salmonellosis as a primary or secondary diagnosis varied slightly year by year, with the lowest number of hospitalisations in 2020 (165; 23.3% of notifications) and highest in 2019 (230 hospitalisations; 19.4% of notifications). The highest percentages of hospitalisations based on case notifications occurred in 2020 (23.3% of notifications) and 2021 (30.4% of notifications), which may have been a consequence of

[26] https://covid19.govt.nz/current-phase-of-our-covid-19-response/; accessed 20 June 2023

only the most severe salmonellosis cases seeking healthcare at a time when there were concerns about healthcare capacity and COVID-19 spread (Imlach et al. 2021).

Deaths associated with salmonellosis are rare. There was one fatality per year associated with salmonellosis from 2005 to 2009, and no fatalities from 2010 to 2014. There was a single death for the time period 2015 to 2021, which occurred in 2017.

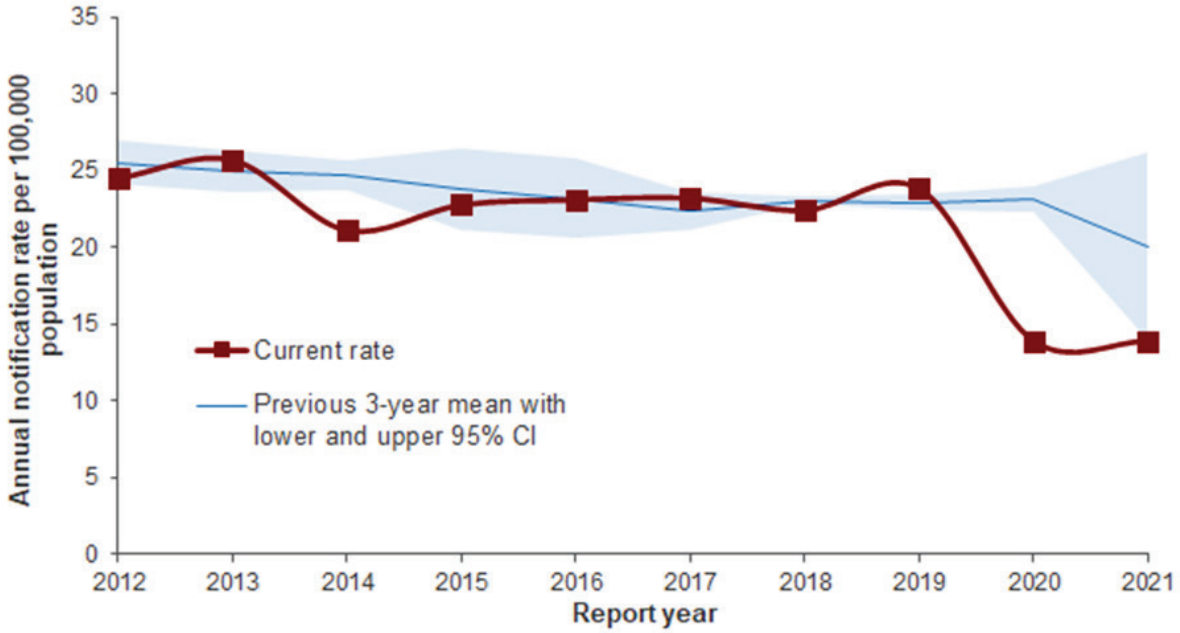

Figure 6. Salmonellosis notification rates by year, 2012-2021. Graph reproduced from Pattis et al. (2022).

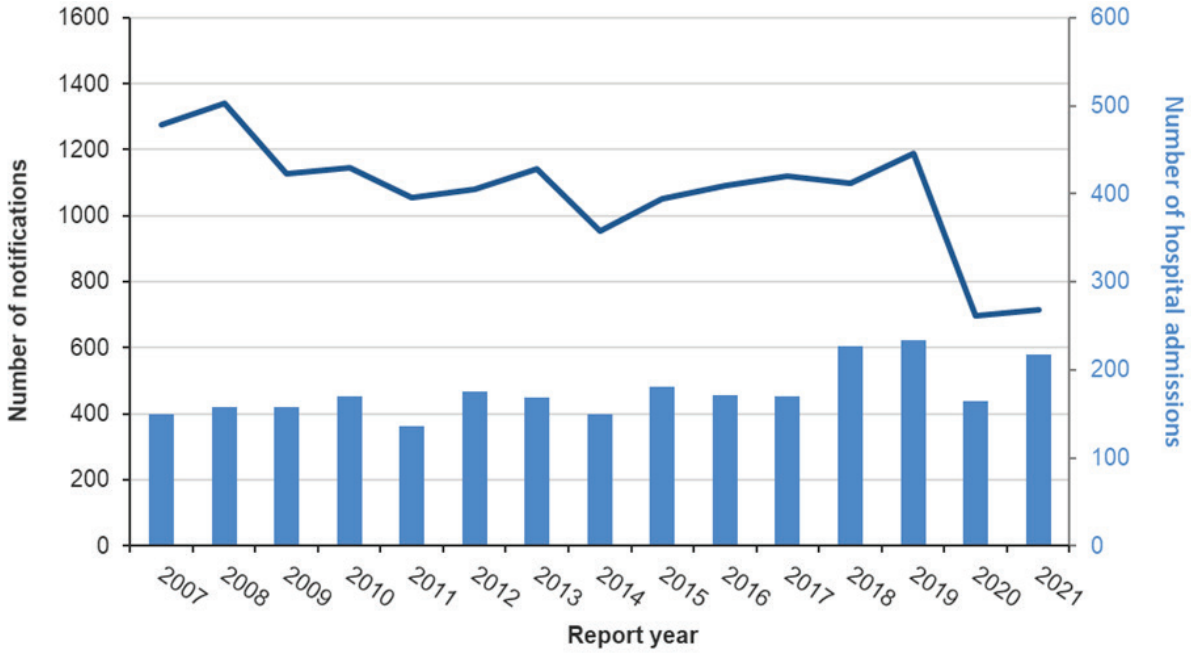

Figure 7. Salmonellosis EpiSurv notifications (line) and Ministry of Health National Minimum Dataset hospitalisations (bar) by year, 2007–2021. Graph reproduced from Pattis et al. (2022).

The incidence of salmonellosis is characterised by a late summer peak and a winter trough. Historically, notification rates have been variable across New Zealand, but the highest rates are often reported from the lower South Island. In 2021 the highest rates were from the lower South Island (29.3/100,000), South Canterbury (24.1/100,000), West Coast (21.4/100,000) and Canterbury (17.1/100,000) (Pattis et al. 2022).

The reported notification and hospitalisation rates were higher for females (14.5 cases per 100,000 population; 4.8 admissions per 100,000 population) than males (13.3 cases per 100,000 population, 339 cases; 3.7 admissions per 100,000 population) in 2021. However, gender proportions differed by year and are generally similar between males and females. Age-specific notification and hospitalisation rates of salmonellosis are consistently highest for the 0 to 4 year age group (52.7 cases per 100,000 population, and 10.8 admissions per 100,000 population in 2021).

3.3.2 Serotypes causing disease in New Zealand

The ESR ERL performs typing of *Salmonella* for the whole of New Zealand. The 2016 Risk Profile (Rivas et al. 2016) reported that of 5,326 serotyped isolates from human salmonellosis cases in New Zealand during the period 2010-2014, 45% were *S.* Typhimurium (mostly DT56 variant) and 12% were *S.* Enteritidis.

Table 7 displays the peak years and total number of cases for serotypes that have caused 50 or more salmonellosis cases between 2015 and 2022. There were 7,910 New Zealand cases of salmonellosis reported for the period 2015 to 2022 for which the *Salmonella* serotype was available[27]. *S.* Typhimurium was the reported cause of 38.2% of these cases and the next most frequently reported serotype was *S.* Enteritidis (12.0% of cases). When considering serotype and phage type, *S.* Typhimurium DT56 variant was the most frequently reported phage type (6.9% of the cases for the years 2015 to 2019; from 1 November 2019, phage typing was discontinued). Following implementation of sequence typing, the most commonly reported ST was *S.* Typhimurium ST19 (554 cases, 16.9% of cases from 2019 to 2022). Together the 17 serotypes listed in Table 7 caused 80.3% (6,356) of the 7,910 cases.

[27] Numbers are from yearly reports (https://www.esr.cri.nz/our-research/nga-kete/infectious-disease-intelligence/enteric-reference-testing/human-salmonella-isolates/; accessed 27 June 2023).

Table 7. *Salmonella* serotypes and phage types that caused 50 or more cases during the period 2015 to 2022 – peak occurrence and total cases.[1]

Serotype / phage type (DT)/ sequence type (ST)[2]	2015	2016	2017	2018	2019	2020	2021	2022	Total isolates 2015-2022
Total typed	*1133*	*1150*	*1217*	*1125*	*1153*	*726*	*668*	*738*	*7,910*
Typhimurium (total)	447	387	432	346	412	334	314	349	*3,021*
DT56 variant	96	64	117	70	49	-	-	-	*396*
DT101	56	47	66	60	36	-	-	-	*265*
DT135	64	30	34	39	21	-	-	-	*188*
RDNC[3]	19	42	44	27	26	-	-	-	*158*
DT108/170	11	22	13	4	83	-	-	-	*133*
DT1	38	34	22	16	7	-	-	-	*117*
DT9	27	42	14	21	13	-	-	-	*117*
DT42	24	12	27	13	11	-	-	-	*87*
DT23	10	8	6	16	17	-	-	-	*57*
DT156	27	12	4	12	1	-	-	-	*56*
ST19	-	-	-	-	36	167	149	202	*554*
ST568	-	-	-	-	26	95	94	98	*313*
ST2297	-	-	-	-	13	68	44	28	*153*
Enteritidis (total)	110	114	150	130	167	72	129	79	*951*
DT11	45	46	55	30	31	-	-	-	*207*
RDNC[3]	20	20	16	15	11	-	-	-	*82*
DT1	17	9	7	9	8	-	-	-	*50*
ST11	-	-	-	-	22	50	59	39	*170*
ST183	-	-	-	-	1	20	65	39	*125*
Brandenburg	52	67	55	45	42	38	37	21	*357*
Bovismorbificans	23	39	52	83	50	58	49	46	*400*
Stanley	25	60	39	35	41	12	9	18	*239*
Saintpaul	37	35	27	39	22	26	31	22	*239*
Infantis	52	14	19	16	26	8	8	7	*150*
Subsp. (I) ser. 4,[5],12:i:-[5]	22	23	28	26	48	0	0	0	*147*
Paratyphi B var Java	21	18	26	32	27	11	3	2	*140*
Mississippi	16	21	15	15	15	15	10	15	*122*
Weltevreden[4]	18	18	21	21	20	11	2	6	*117*
Thompson	32	13	12	10	9	15	11	13	*115*
Agona	12	18	16	27	14	4	4	10	*105*
Newport	14	22	20	10	9	4	1	2	*82*
Virchow	16	10	7	7	8	4	0	9	*61*
Javiana	5	11	18	6	5	1	2	8	*56*
Kentucky	11	10	15	8	9	0	1	0	*54*

[1] Data are from reports available from https://surv.esr.cri.nz/enteric_reference/human_salmonella.php (accessed 1 March 2023).

[2] From 1st November 2019, ESR replaced phage typing (DT) of *S.* Typhimurium and *S.* Enteritidis with whole genome sequencing (WGS) which returns a sequence type (ST). ST does not relate to DT.

[3] *S.* Enteritidis RDNC and *S.* Typhimurium RDNC are not single serotypes, but a grouping of serotypes. RDNC stands for 'reaction does not conform' and indicates that the isolate does not match any recognised serotypes.

[4] Weltevreden also includes ST365 and var. 15+.

[5] Following the introduction of WGS, *Salmonella* Subsp. (I) ser. 4,5,12:i:- is now reported as monophasic *S.* Typhimurium.

3.3.3 Antimicrobial resistance of New Zealand *Salmonella* strains

Hospital and community laboratories are requested to refer all *Salmonella* isolates from human salmonellosis cases to ESR as part of the laboratory-based surveillance. *Salmonella* from other sources, including food, animal and environmental sources, are also referred to ESR for epidemiological typing. The ESR Antibiotic Reference Laboratory also tests the antimicrobial susceptibility all isolates of phage types that were internationally recognised as being multidrug-resistant. The antimicrobial susceptibility of a representative sample (approximately 20%; every fifth isolate received) of non-typhoidal isolates was tested yearly until 2016, and again in 2019.

Results for antimicrobial resistance testing for the years 2015 to 2019 are compiled in Appendix B.1. The most recent report states that resistance remains relatively low, with 91.0% fully susceptible to all 11 antimicrobials (89.3% of human isolates and 93.1% of non-human isolates) (ESR 2019). This is similar to data from 2014 where 86% of isolates remained fully susceptible; the range in susceptibility from 2015 to 2019 was 89.3% to 91.0%. Note that the panel of antimicrobials tested differed slightly across years. The susceptibility to 14 different antibiotics were tested in total across all years; susceptibility to 11 antibiotics was tested each year.

For the time period 2015 to 2019, *Salmonella* isolates from salmonellosis cases reported to have travelled overseas were significantly ($p < 0.05$) more resistant to at least one antimicrobial than isolates from cases for whom no recent overseas travel was reported.

No data were found after 2019 on antimicrobial resistance of New Zealand *Salmonella* isolates from layer chickens or eggs. However, as discussed in Appendix A.3, New Zealand takes a conservative approach to the use of antibiotics in poultry farming, and their usage is lower than other countries. In other countries, resistance of *Salmonella* isolates from eggs to a range of different antimicrobials has been reported (Appendix B.1.1).

3.3.4 Reported New Zealand outbreaks

The number of reported salmonellosis outbreaks and case numbers, including those reported as foodborne and where eggs were listed as a suspected source, are shown in Table 8.

Over the period 2015 to 2021, the annual number of salmonellosis outbreaks with food reported as a possible mode of transmission ranged from two (2020) to 15 (2019). The total number of cases associated with these outbreaks ranged between 15 (2017) and 186 (2019).

Table 8. Reported salmonellosis outbreaks in New Zealand and information on those reported as foodborne (2015-2021).

Year	Salmonellosis outbreaks	Cases associated with salmonellosis outbreaks	Salmonellosis outbreaks reported as foodborne (number of cases)[1]	Foodborne salmonellosis outbreaks where eggs implicated	Reference
2015	18	101	3 (30)	0	(Lopez et al. 2016)
2016	24	130	12 (78)	1	(Pattis et al. 2017)
2017	13	40	4 (15)	1	(Pattis et al. 2019b)
2018	14	75	5 (17)	0	(Pattis et al. 2019a)
2019	27	226	15 (186)	3	(Pattis et al. 2020)
2020	8	34	2 (12)	0	(Horn et al. 2021)
2021	8	99	5 (90)	1	(Pattis et al. 2022)
Total	***112***	***705***	***46 (428)***	***6***	

[1]An outbreak is classed as foodborne if food was recorded as one of the likely modes of transmission applicable to the outbreak. Single outbreaks may have multiple pathogens, modes of transmission, settings where the exposure occurred, or settings where preparation of food was conducted. Other modes of transmission may also be reported.

3.3.5 Egg consumption as a risk factor for salmonellosis in New Zealand

From an expert elicitation carried out in 2013, the estimated proportion of salmonellosis in New Zealand that is due to foodborne transmission was 62.1% (95th percentile credible interval 35.2-86.4%, based on self-assessed performance weighting) (Cressey et al. 2019). The proportion of foodborne transmission due to eggs was not considered.

There were six outbreaks for the period 2015 to 2021 where eggs or egg products were listed as a suspected or confirmed source of infection (Table 8, Table 9). This represents 13% of the outbreaks that were reported to be foodborne. Further detail on these outbreaks is provided in Table 9. There were 103 (79 confirmed and 24 probable) cases associated with the six outbreaks. The confirmed cases represent 18% of cases from outbreaks reported as being foodborne, and 11% of all cases associated with salmonellosis outbreaks. There was a single outbreak where the evidence for the consumption of eggs was strong, although poultry meat was also considered a potential source. This 2021 *S*. Enteritidis DT8, ST11 outbreak is covered in more detail in the following section.

For the period 2010 to 2014, there were three outbreaks where eggs or egg products were a suspected source (Rivas et al. 2016). Two were considered to have strong evidence for egg as the vehicle of infection. These included a chocolate mousse cake made with raw eggs served at a café/delicatessen (involving *S*. Typhimurium DT155; 10 confirmed cases, 11 probable cases, 44 people exposed), and a boiled egg and ham sandwich served at a café/bakery (involving *S*. Infantis; 10 confirmed cases).

There have been no new case control studies since the 2016 Risk Profile (Rivas et al. 2016) evaluating eggs as a risk factor for salmonellosis in New Zealand. There have also not been any recent source attribution studies for *Salmonella* in New Zealand.

Table 9. New Zealand non-typhoid salmonellosis outbreaks where eggs were a suspected or confirmed source of infection, 2015-2021.

Year[1]	*Salmonella* serotype	Food(s) reported	Exposure setting	Number of cases[2]	Strength and type of evidence[3]	Reference
2016	Not provided	Eggs, raw peppers, soft brie cheese	Supermarket/ delicatessen	3C 0P	Weak evidence for food source	(Pattis et al. 2017)
2017	Not provided	Raw eggs, chicken, untreated water	Marae	4C 1P	Weak evidence for food source	(Pattis et al. 2019b)
2019	*S.* Typhimurium DT56 variant	Undercooked old eggs	Home	2C	Common meal; weak evidence for food source	(Pattis et al. 2020)
	S. Enteritidis DT21	Eggs Benedict	Overseas restaurant/café/bakery	7C, 2P	Consumption of same food type; weak evidence for food source	
	S. Enteritidis ST11	Desserts prepared by infected food handler[4]	Restaurant/café/bakery	17C, 21P	Weak evidence for food (at the time). Common food premise and food type, epidemiological investigation	
2021	*S.* Enteritidis ST11	Poultry[5]	Work, home or restaurant/café/ bakery	46C	WGS of case isolates matching those found in poultry flocks	(Pattis et al. 2022)

[1] Based on the date of onset of symptoms in the index case in the outbreak.

[2] C, confirmed cases; P, probable cases.

[3] The strength and type of evidence is not always provided. Outbreaks with strong evidence included those with a statistically significant elevated risk ratio or odds ratio (95% confidence) from an epidemiological investigation and/or laboratory evidence with the same organism and strain detected in both disease cases and vehicle (to the highest available level of identification). Outbreaks with weak evidence met one or more of the following criteria: compelling evidence with symptoms attributable to specific organism, other association but no microbial evidence for causal link i.e. organism detected at source but not linked directly to the cases by indistinguishable DNA or PFGE profiles, raised but not statistically significant relative risk or odds ratio, or no evidence found but logical deduction given circumstances.

[4] This outbreak involved the same *S.* Enteritidis ST11 strain as in the 2021 outbreak. The source in this 2019 outbreak was later considered likely to have been the eggs used in a raw egg dessert.

[5] The number of confirmed cases in the table are those that were part of a distinct temporal cluster of cases in 2021, with illness onset dates from 3 February 2021 to 29 June 2021. Additional cases from 2019 to 2022 have also been linked by WGS, for example the 2019 outbreak with *S.* Enteritidis DT8. In the 2021 cluster, the outbreak strain was also detected in samples from hatchery, layer and broiler poultry flocks. Person-to-person transmission was reported as a possible risk factor for six of the cases and some of the cases were reported as working on poultry farms.

3.3.6 2021 *S.* Enteritidis DT8, ST11 outbreak (SE 2021) associated with poultry meat and eggs

On 25 February 2021, ESR serotyped an isolate from a raw broiler chicken carcass, sampled on 17 February 2021 during routine NMD programme testing from a large-scale poultry meat processor, as *S.* Enteritidis (Jackie Wright, ESR, pers. comm). The isolate was entered into the NMD database on 3 March 2021. This represented the first detection of this serotype from New Zealand poultry. On 19 March 2021, MPI was informed that following WGS, the ST11 isolate formed a close genomic cluster (<5 SNPs) with an ongoing cluster of human cases from multiple Public Health Units from the North Island, predominantly the Auckland region, that dated back to 2019 (Ministry for Primary Industries 2021, Pattis et al. 2022). The outbreak strain (designated as *S.* Enteritidis genomic cluster profile *S.* Enteritidis_2019_C_01) was

subsequently identified at additional poultry operations, one of which was a major supplier of day-old chicks and hatching eggs for the poultry meat and egg industries in New Zealand, and the other a rearer of pullets for egg-laying (Ministry for Primary Industries 2021). Both the initial broiler and rearer detections were from farms supplied by the hatchery on the same day (19 January 2021). The working assumption was that the hatchery was the source of infection in downstream operations and therefore further infections within connected poultry producers (egg laying, broiler, or rearer) were likely.

The timeline and number of cases notified with the outbreak strain are shown in Figure 8. As of 30 May 2023, there have been 128 confirmed outbreak cases (person notified in New Zealand with the *S*. Enteritidis genomic cluster profile *S*. Enteritidis_2019_C_01), as well as an additional six cases that were epidemiologically linked; totalling 134 cases. The earliest case was from May 2019, and the report date of the most recent case was 3 February 2023. The proportion of cases hospitalised with the outbreak strain was 36.6% (49/134 cases) (EpiSurv data; Shevaun Paine, pers. comm, 9 May 2023). The hospitalisation rate was higher than for all salmonellosis cases (792/2889 cases; 27.4%) or total *S*. Enteritidis cases (112/397 cases; 28.2%) over a similar reporting period. The outbreak was not considered to have materially affected the total number of salmonellosis cases reported for 2021 (Pattis et al. 2022).

Most of the isolates comprising the outbreak strain from cases and the poultry environment were phage typed as DT8. Isolates from some cases were originally typed as DT28, but later retyped as DT8 during the outbreak response. DT28 reacts with the same phages as DT8 but the reaction intensity with phages 3, 7 and 11 are much weaker (Jackie Wright, ESR; pers. comm; 16 July 2021). Phage types DT2 and DT23 were also identified amongst genomically linked poultry isolates. Isolates of DT8 and DT28 have been reported to be potentially capable of transovarian contamination of eggs in international studies (Thiagarajan et al. 1994, Thiagarajan et al. 1996, Dawoud et al. 2011), and DT8 was previously reported to be the predominant phage type of *S*. Enteritidis from both human outbreaks and poultry flocks in the United States (Altekruse et al. 1993, Denagamage et al. 2016). In addition, all isolates were ST11 which is the most common sequence type of *S*. Enteritidis internationally (Luo et al. 2021), and which has been implicated in large outbreaks associated with both eggs and poultry meat internationally (European Centre for Disease Prevention and Control and European Food Safety Authority 2021, 2022a).

Multiple lines of evidence supported that poultry meat and/or eggs were the most likely source of the outbreak (Jefferies et al. 2021, Ministry for Primary Industries 2021, French et al. 2022, Pattis et al. 2022). These included:

- There was a very high degree of similarity between all SE_2019_C_01 isolates from poultry and clinical sources (<5 SNP differences). This is consistent with transmission between poultry and humans, most likely through the food chain.
- The epidemiology of human salmonellosis cases infected with SE_2019_C_01 detected in New Zealand from 2019-2021 is consistent with a foodborne outbreak associated with multiple contaminated poultry and/or egg exposure pathways with amplification events which themselves can be (and have been) defined as outbreaks (see Figure 8).

- Of the SE 2019_C_01 cases that presented late 2021 to early 2022 that were interviewed by a Public Health Unit-administered supplementary questionnaire, the majority (10/11) reported having some exposure to poultry meat and/or eggs during their incubation period for disease. The remaining case possibly had exposure through home-made mayonnaise; however, recall bias was a factor in the case interview. One case was a maintenance worker at the hatchery from which the *S*. Enteritidis outbreak strain was detected, who possibly had contact with chicken faeces on the shed floor where chickens lay eggs. As consumption of poultry meat and eggs are common in the general population, it is difficult to interpret this finding in isolation. The small case numbers and limitations in collecting dietary histories, including biases and resourcing required to implement a detailed dietary questionnaire, have prevented further epidemiological analysis. The timelines of case detection also limits the acquisition of food products consumed by cases for testing. However, the food histories of recent cases do continue to identify poultry and/or eggs as plausible sources of ongoing exposure to this pathogen.
- There was an increase in human cases concomitant with poultry meat from the flock that tested positive during NMD programme testing being released onto the market for consumption (Figure 8).
- The outbreak strain was detected from 2020 and 2021 samples from the layer farm that supplied eggs to a restaurant involved in a 2019 outbreak associated with a raw egg dessert.
- The majority of isolates of the outbreak strain were DT8, which might contaminate the yolk of eggs through transovarian transmission, and therefore increase the risk to human health through consumption of uncooked or undercooked egg yolks. Overseas experience shows that *S*. Enteritidis typed as DT8 or ST11 can cause substantial outbreaks of human salmonellosis associated with poultry meat and or eggs.

Following the notification of *S*. Enteritidis detection in poultry on 19 March 2021, MPI launched response and regulatory activities (Ministry for Primary Industries 2021) which included:

- A historical/traceback phase to investigate earlier detections of the outbreak strain (covering the period May 2019 to 19 March 2021);
- An investigative phase to determine the scope of the outbreak (19 March to 22 April 2021);
- A delimiting phase to understand the prevalence and risks associated with the outbreak strain across commercial layer flocks (22 July 2021 to 10 September 2021);
- An emergency control scheme (ECS) to temporarily regulate the poultry production supply chain and manage risks to public health and international trade of *S*. Enteritidis, which came into effect on 6 October 2021.
- Targeted consultation with the poultry industry on proposed regulatory options for long term management of *S*. Enteritidis in early 2022, with a recommendation to the Minister for poultry producers to operate under an RMP.
- Long term regulations which came into force 6 October 2022, requiring poultry producers to operate under an RMP no later than 1 November 2023. Implemented management programmes are covered in Section 4.

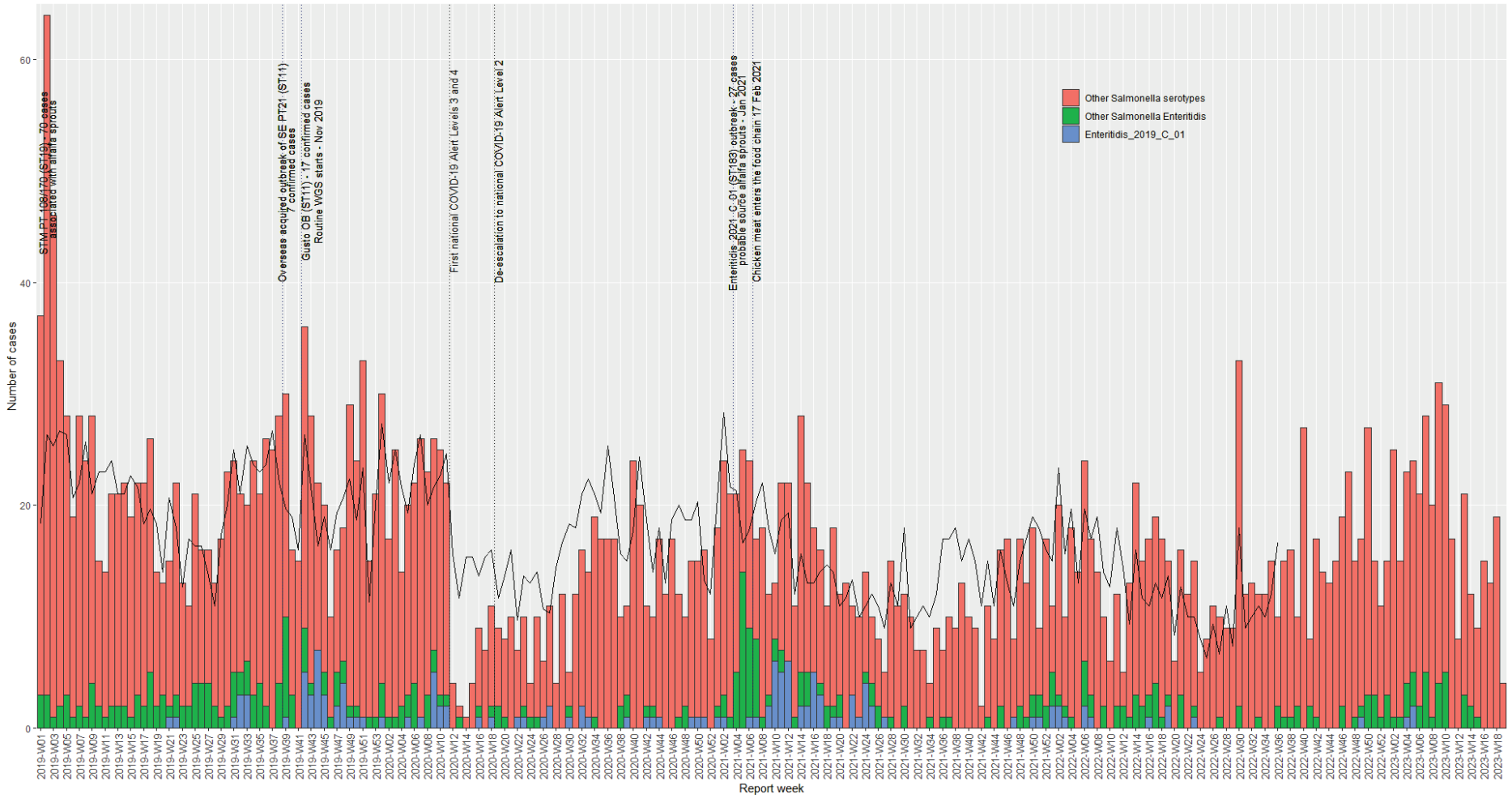

Figure 8. Salmonellosis, *S.* Enteritidis and *S.* Enteritidis_2019_C_01 case notifications by report week, Jan 2019 to May 2023. Data source: EpiSurv as at 0900hrs 09 May 2023.

3.4 SALMONELLOSIS IN OTHER COUNTRIES

Key findings

- New Zealand notified salmonellosis rates for 2018 to 2021 (13.9 to 24.3 cases per 100,000 population) were similar to rates from the EU (13.7 to 20.1 cases per 100,000 population), and lower than Australian rates (41.7 to 57.5 cases per 100,000 population). Rates were higher than reported in the US for 2018 and 2019, but similar during 2021 and 2022 where the COVID-19 response affected reporting levels in both countries.
- Chicken eggs and chicken meat were commonly identified as an important vehicles of foodborne disease in other countries. However, relative proportions of total disease incidence depend on the country or region, and the predominant *Salmonella* serotype/s associated with layer flocks. The overwhelming majority of egg-associated outbreaks in the US, Canada and Europe were caused by *S*. Enteritidis, compared with *S*. Typhimurium in Australia.

3.4.1 Incidence of salmonellosis in other countries compared with New Zealand

The incidence of notified cases of salmonellosis in New Zealand for the years 2018 to 2021 is similar to rates in other developed countries and regions, particularly for the EU (13.7 to 20.1 cases per 100,000 population) (Table 17, Appendix). Annual rates of salmonellosis for these years in Australia (41.7 to 57.5 cases per 100,000 population) were higher than New Zealand rates, which was also reported in the 2016 Risk Profile (Rivas et al. 2016). In Australia, salmonellosis rates have been significantly increasing since national notifications began in 1991, which was attributed in part to the increase in PCR-based testing for laboratory diagnosis since 2014 (OzFoodNet Working Group 2021b).

As was reported for New Zealand, the annual incidence of salmonellosis was lower during 2020 and 2021 relative to 2019 for all regions for which data was reported in Table 17, due to the impact of the COVID-19 pandemic. In 2018 and 2019, New Zealand rates of salmonellosis were slightly higher than in the US. Specifically, there were 22.5 and 24.3 cases per 100,000 population in New Zealand compared with 18.3 and 17.1 cases per 100,000 population in the US, for 2018 and 2019, respectively. However, there was a bigger reduction in salmonellosis incidence in New Zealand due to COVID-19 than was observed in the US. This resulted in more similar rates between New Zealand and the US in 2020 and 2021 (13.9 cases per 100,000 population in New Zealand for both years compared with 13.3 and 14.2 cases per 100,000 population in the US for 2020 and 2021, respectively).

The proportions of *Salmonella* serotypes observed from cases varies considerably based on country. As in New Zealand, the most common serotype from cases in Australia was *S*. Typhimurium. In contrast to New Zealand, the dominant serotype in the EU, US and Canada was *S*. Enteritidis.

3.4.2 Health burden of salmonellosis in New Zealand and internationally

The most recent update of the estimate of the burden of foodborne disease for New Zealand, which is based on surveillance data for 2013, includes an estimate for foodborne salmonellosis of 74 disability adjusted life years (DALYs)[28] (Cressey and Lake 2014). This placed foodborne

[28] The calculation for DALYs is the number of years of life lost to mortality combined with the number of years lived with disability.

salmonellosis fifth on the list for foodborne disease burden (after norovirus infection, campylobacteriosis, listeriosis and Shiga toxin-producing *Escherichia coli* infection). The New Zealand estimate of the burden of foodborne disease from salmonellosis does not subdivide the burden according to specific foods. The estimate does not include a monetisation of the burden of disease.

An expert elicitation study conducted by the WHO Foodborne Disease Burden Epidemiology Reference Group Source Attribution Task Force has also estimated the relative contribution of food to the global burden of non-typhoidal salmonellosis and other predominantly foodborne pathogens. The foodborne transmission route was considered more important in the developed subregions (America, Europe and the Western Pacific) compared with developing subregions (African, American and Eastern Mediterranean region). In the developing subregions, there were relatively more contributions from other routes (animal contact, water and soil). For the Western Pacific region (that included New Zealand), the proportion of salmonellosis acquired through foodborne transmission was estimated at 0.74 (95% uncertainty interval 0.45-0.93) (Hald et al. 2016).

The Global Burden of Diseases, Injuries, and Risk Factors Study 2017 (Stanaway et al. 2019) estimated that *Salmonella* enterocolitis resulted in 95.1 million cases (95% uncertainty interval: 41.6-184.8), 50,771 deaths (2824-129,736), and 3.10 million DALYs (95% uncertainty interval: 0.39-7.39) in 2017. Furthermore, the global burden of invasive salmonellosis for 2017 has been estimated to be 4,263,500 DALYs (95% uncertainty interval: 2,384,900 to 7,382,000), or a rate of 616,800 (95% uncertainty interval: 347,300 to 1,076,200) per million people. The DALY rates in the Southeast Asia, east Asia and Oceania super-region were calculated at 49,900 (95% uncertainty interval: 27,400-87,600) per million people. The highest rate of invasive salmonellosis was in sub-Saharan Africa with 2,687,700 (95% uncertainty interval: 1,495,400-4,552,800) DALYs per million people.

A 2017 Belgian study estimated and forecasted the burden of salmonellosis and other foodborne diseases (campylobacteriosis and listeriosis) from 2012 to 2020 (Maertens de Noordhout et al. 2017). The calculations were based on a Belgian population of 11.2 million people in 2012 and a predicted a population of 11.4 million people in 2020. The estimated DALYs for salmonellosis were 102 (95% uncertainty interval: 8-376) in 2012, or 0.9 DALYs per 100,000 population (95% uncertainty interval: 0.07-3). These were predicted to drop to 82 (95% uncertainty interval: 6-310), or 0.7 DALYs per 100,000 population (95% uncertainty interval: 0.05–3), in 2020. The estimated drop takes into account the trend of decreasing salmonellosis cases that occurred after 2005, when Belgium adopted changes to poultry monitoring and controls, and introduced poultry vaccination against *Salmonella*. This resulted in a dramatic decreases in salmonellosis caused by *S*. Enteritidis, while the number of cases caused by other serotypes remained constant.

An Australian study estimated that the total cost of salmonellosis and its sequelae was AUD 140 million per year (Australian National University 2022). The estimate was based on 2019 data of an estimated 61,600 cases of foodborne salmonellosis, 3,740 hospitalisations and 11 deaths. The costing was also based on an estimated 5,750 cases and 172 hospitalisations

due to reactive arthritis following salmonellosis and 5,400 cases and 460 hospitalisations due to irritable bowel syndrome following salmonellosis. DALYs were not reported.

In the EU, EFSA has estimated that the overall economic burden of human salmonellosis may be as high as EUR 3 billion per year.[29]

3.4.3 International source attribution studies

International studies have used expert opinion, statistical modelling, typing data and outbreak reports to attribute salmonellosis to sources or vehicles of infection. Chicken eggs were commonly identified as an important source of foodborne disease. However, relative proportions depend on multiple variables such as the country or region, the *Salmonella* serotype/s analysed, and the methods used. A summary of recently published salmonellosis attribution studies from different countries that considered eggs, is included below.

Australia: Chickens (both broilers and layers) were identified to be the primary source of salmonellosis in a number of Australian attribution studies. Note that much of the data from Australian source attribution studies covered a time period when *S*. Enteritidis was not present in poultry flocks. In New South Wales (NSW), layer chickens were the primary reservoir of domestically acquired *Salmonella* infections (McLure et al. 2022). In South Australia, 37% of sporadic cases and 59% of outbreak-related cases were attributed to chicken eggs (Glass et al. 2016). In Queensland, 65.3% of cases were attributed to either chicken meat or eggs (Fearnley et al. 2018), while another study reported 4.8% attribution to poultry during 2008–2012 and 24% in 2013–2017 (Munck et al. 2020). In the study by Fearnley et al. (2018), the serotypes most commonly associated with chicken and eggs were *S.* Typhimurium, *S*. Anatum, *S*. Enteritidis and *S*. Saintpaul.

Europe: Between-country differences in epidemiology were observed. Depending on the study and country, pigs or layer chickens/eggs were the most important contributors to human salmonellosis, with lower contributions from broiler poultry and poultry meat (Mughini-Gras et al. 2014a, Mughini-Gras et al. 2014b, De Knegt et al. 2015, Merlotti et al. 2020). Pigs were identified as the main contributor to salmonellosis due to the serotype *S*. Typhimurium, while the majority of *S*. Enteritidis infections were attributed to laying hens/eggs followed by broilers and turkeys (Mughini-Gras et al. 2014b, De Knegt et al. 2015, Merlotti et al. 2020, Arnold et al. 2021). In 2021, *S*. Enteritidis remained the most frequently reported causative agent of foodborne outbreaks as also reported in previous years, and *Salmonella* in 'eggs and egg products' was listed as the agent/food pairing of most concern (European Food Safety Authority and European Centre for Disease Prevention and Control 2022).

US: Based on a model using 2020 US outbreak data from the US Centers for Disease Control and Prevention (CDC), the largest proportion of salmonellosis cases (approximately 17.3% of the estimated one million annual non-typhoidal salmonellosis cases) in the US have been attributed to chicken meat products (The Interagency Food Safety Analytics Collaboration 2022). Of the 16 other food categories listed, the next seven categories included: fruits (14.9%), pork (12.8%), seeded vegetables (12.0%), other produce (8.6%), beef (6.0%), turkey

[29] https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/factsheetsalmonella.pdf; accessed 6 January 2023

meat (5.9%) and eggs (5.7%). Salmonellosis attribution to chicken eggs in the US appears to be decreasing, with estimates from previous years including 6.3% in 2019, 6.9% in 2018, 7.9% in 2017 and 2016, and 12% in 2012.[30]

3.4.4 International outbreaks associated with eggs

Recent human outbreaks in other countries have been attributed to the consumption of eggs and egg products contaminated by a number of different serotypes (Table 18, Table 19; Appendix). However, the overwhelming majority of outbreaks in the US, Canada and Europe were caused by *S*. Enteritidis, compared with *S*. Typhimurium in Australia. There have also been various recalls of eggs and egg product due to contamination with *Salmonella,* especially by *S*. Enteritidis (Table 20; Appendix).

[30] https://www.cdc.gov/foodsafety/ifsac/annual-reports.html;. Accessed 11 January 2023

4 REGULATORY CONTROLS

Key findings

- Detection of *S*. Enteritidis in New Zealand poultry flocks has driven the development of a new regulatory framework for all sectors within the poultry industry. New requirements include, but are not limited to: the development of a Risk Management Programme for all sectors of the industry; microbiological testing of the poultry environment for *S*. Enteritidis; procedures for the tracing and elimination of *S*. Enteritidis from the poultry supply chain where detected; and changes to Overseas Market Access Requirements to manage risks posed by *S*. Enteritidis to international trade of poultry product.
- International control measures and testing programmes for *Salmonella*, including for *S*. Enteritidis, in the egg layer industry vary between countries. New Zealand testing requirements for *S*. Enteritidis in the egg layer production environment are at least as stringent as those used internationally with respect to sample types, numbers, frequency and timing of testing. However, the EU and UK regulatory controls additionally target *S*. Typhimurium in all flocks, as well as three other serotypes in breeder flocks.

4.1 CURRENT CONTROL AND RISK MANAGEMENT MEASURES

4.1.1 Management of *S*. Enteritidis: a regulatory framework

Considerable changes to *Salmonella* control and risk management measures have been made since the 2016 Risk Profile (Rivas et al. 2016). Following the detection of *S.* Enteritidis from New Zealand poultry environments in March 2021 and following extensive investigation, a new regulatory framework for the industry has been designed and implemented. The *Animal Products Order: Emergency Control Scheme - Managing* Salmonella *Enteritidis in Commercial Chicken Flocks (ECS)* was signed on 6 October 2021.[31] The key components of the ECS were to identify *S*. Enteritidis, and facilitate its management, monitoring and verification (Ministry for Primary Industries 2022f). The ECS temporarily covered a regulatory gap present at early stages in the poultry chain; including at breeding flocks, hatcheries, rearer farms for future layer birds, layer flocks and broiler farms. The ECS was extended in April 2022 for a further six months and expired on 5 October 2022.

The ECS was replaced by a *S*. Enteritidis regulatory framework to manage long-term risks to public health and international trade from *S*. Enteritidis. This was released under an updated *Animal Products Regulations 2021*,[32] which came into effect on 6 October 2022 (described below). The purpose of the *S*. Enteritidis management framework is to detect, manage and assist industry to manage *S*. Enteritidis in commercial poultry flocks as part of routine operations (Ministry for Primary Industries 2022h).

4.1.2 Current legislations and codes

The New Zealand egg layer industry is currently regulated by the following food-related legislations and codes.

[31] https://gazette.govt.nz/notice/id/2022-sl1236; accessed 13 January 2023
[32] https://legislation.govt.nz/regulation/public/2021/0400/latest/LMS520972.html; accessed 13 January 2023

Agricultural Compounds and Veterinary Medicines Act 1997

The purpose of the *Agricultural Compounds and Veterinary Medicines Act 1997*[33] is to prevent or manage risks associated with the use of agricultural compounds. The Act details definitions and requirements for vaccinations, medicines and feeds, which are documented under the Whole Flock Health Scheme. Poultry feed is classed as an “agricultural compound” under the Act and must be authorised. Requirements for poultry feed include complying with minimum manufacturing requirements, ensuring that feed is fit for purpose, and that feed is not misrepresented as anything other than animal feed.

The Animal Products Act 1999

The *Animal Products Act 1999* regulates the processing of animal material into products for use, trade, and export through managing associated risks and facilitating overseas market access.[34] The Act requires all animal products traded and used to be "fit for intended purpose". For poultry producers and processors, this involves requiring that the production and processing of animal materials and products occurs under an RMP (Part 2 of the Act).

Part 3 of the Act provides for the setting of regulated control schemes where risk factors cannot be managed under risk management programmes, or where special provision is required for overseas market access.

Part 4 of the Act provides for the setting of standards that must be met before an animal product can be considered fit for intended purpose, and for the setting of any specifications necessary to ensure the standards are met.

The Act gives MPI the power to issue notices. Notices cover a range of legal requirements for businesses producing, processing, selling, storing, transporting, importing, and exporting animal (and dairy) products.

Animal Products Regulations 2021

The redesigned *Animal Products Regulations 2021*[35] was made under the *Animal Products Act 1999* and came into force on 6 October 2022. It replaced six Regulations; those relevant to the egg layer industry included the *Animal Products Regulations 2000*; *Animal Products (Risk Management Programme Registration – Required Part) Regulations 2020*; *Animal Products (Exemptions and Inclusions) Order 2000*; and *Animal Product (Regulated Control Scheme - Contaminant Monitoring and Surveillance) Regulations 2004*. The purpose of the redesign was to simplify and consolidate existing Regulations under the Act. The Regulations set out animal product standards and provide for the setting of specifications, including new requirements for the egg layer industry. Note that the roles do not apply to people that farm 100 chickens or fewer, or who sell chickens or fertile eggs direct to the consumer or end user.

[33] https://www.legislation.govt.nz/act/public/1997/0087/latest/whole.html; accessed 13 January 2023
[34] https://www.legislation.govt.nz/act/public/1999/0093/latest/DLM33502.html; accessed 13 January 2023
[35] https://legislation.govt.nz/regulation/public/2021/0400/latest/LMS520972.html; accessed 13 January 2023

The *Animal Products Notice: Production, Supply and Processing* was issued under the *Animal Products Act 1999* for the purpose of supplementing the *Animal Product Regulations 2021* (Ministry for Primary Industries 2022c). Relevant to the egg production industry, it covers:

- Requirements for breeders, hatcheries, rearers, egg producers and processors to have an RMP;
- Requirements for egg processors, such as monitoring of treated water used in egg processing (microbiology – *E. coli*/coliforms, turbidity, pH and chlorine), handling of eggs from *S*. Enteritidis-positive flocks (termed "*S*. Enteritidis-positive eggs"), and egg processing;
- New environmental sampling, routine sampling and laboratory testing requirements for *S*. Enteritidis;
- Requirements when *S*. Enteritidis is detected;
- Good Operating Practice, including: for premises and equipment; cleaning and maintenance; pest control and exclusion of animals; personnel, contractors and visitors; feed management; additional requirements for producers of breeder chickens and day-old chicks.
- Competencies of personnel training, including sampler training and managing *S*. Enteritidis requirements; and
- Operator verification for chicken producers.

Animal Welfare Act 1999

The *Animal Welfare Act*[36] defines the fundamental obligations for how people should care for and act towards animals. The *Code of Welfare: Layer Hens (2018)* (Ministry for Primary Industries 2018b) was issued under the *Animal Welfare Act 1999* by the National Animal Welfare Advisory Committee. The Code was since amended by the *Animal Welfare (Care and Procedures) Amendment Regulations (2020).*[37] The Code of Welfare expands on the basic obligations of the Act by setting minimum standards and recommending best practice for the care and management of animals.

The *Code of Welfare: Layer Hens (2018)* is intended for all people responsible for the welfare of layer chickens. It applies to all layer hens from the time chicks are in the last half of development before they hatch (which has relevance to the sale of embryonated eggs), through to the catching and transport of hens at the end of the laying cycle. It also applies to roosters, but does not apply to layer hen breeder birds. Aspects covered by the Code include: stockmanship, feed and water, shelter and facilities, providing for behavioural needs, physical handling, disease and injury control, humane destruction, hatchery management, and a welfare assurance system. A particular feature of the new Code is that conventional cages will be phased out by the end of 2022 (as also discussed in Section 2.2.2).

There are no specific standards for *Salmonella*, but many of the requirements will help control it, for example:

- premises and equipment must be thoroughly cleaned before restocking to prevent the carry-over of disease-causing organisms to incoming hens,

[36] https://www.legislation.govt.nz/act/public/1999/0142/latest/DLM49664.html; accessed 13 January 2023
[37] https://www.legislation.govt.nz/regulation/public/2020/0172/latest/LMS329846.html; accessed 13 March 2023

- manure removal under cages,
- prevention of induced moulting and handling methods that minimise stress,
- measures to control pests in and around hen housing and shelters, and
- litter management to avoid diseases (such as minimising events that result in wet litter and replacement after every laying cycle).

The Food Act 2014

The Food Act 2014[38] was introduced to ensure that food sold through New Zealand is safe and suitable, and provides for more stringent food safety requirements for higher risk food businesses. Relevant to the egg production industry, the Act requires secondary processors that break eggs and make egg products (and not operating under an RMP) to operate under a Food Control Plan, which is a written plan for managing food safety on a day-to-day basis.

Provisions of the *Australia New Zealand Food Standards Code*[39] have been adopted under the *Food Act 2014*. Chapter 1 of the Code contains requirements that are applicable to the egg production industry (for example, Standard 1.2 lists requirements for labelling, and Standard 1.3 lists substances that can be added to foods such as processing aids used to wash eggs). Standard 1.6 and Schedule 27 set out the microbiological limits for specific food products.[40] For all processed egg product, which refers to egg product that has been pasteurised or subjected to an equivalent treatment, *Salmonella* must not be detected in five 25 g samples of food from the same lot. There are no standards for *Salmonella* in or on whole eggs.

4.1.3 Mandatory requirements and voluntary controls

Risk Management Programmes

The *Animal Products Act 1999*[41] defines an RMP as a programme designed to identify and control, manage, and eliminate or minimise hazards and other risk factors in relation to the production and processing of animal material and animal products in order to ensure that the resulting animal product is fit for intended purpose. An RMP is based on the principles of Hazard Analysis and Critical Control Point (HACCP): identifying the hazards, the systems of control, and demonstrating that the controls are effective (Ministry for Primary Industries 2022j). The Act requires that RMPs are tailored for each animal product business according to the animal materials used, the processes performed and the product range produced. Operators must build any relevant regulatory limits (for example, microbiological limits) into their RMP, but can also set their own measurable limits to ensure the food is safe and fit for purpose.

As discussed in the 2016 Risk Profile (Rivas et al. 2016), egg layer farms and primary processors who harvest, candle, grade, and pack eggs, have been required to have a registered RMP under the *Animal Products Act 1999*. Secondary processors of eggs could choose to operate under an RMP or a Food Control Plan (as discussed above). RMPs (or

[38] https://www.legislation.govt.nz/act/public/2014/0032/75.0/DLM2995811.html; accessed 13 March 2023
[39] https://www.foodstandards.gov.au/code/pages/default.aspx; accessed 13 March 2023
[40] https://www.legislation.gov.au/Details/F2021C00605l; accessed 13 March 2023
[41] https://www.legislation.govt.nz/act/public/1999/0093/latest/whole.html; accessed 13 March 2023

Food Control Plans for secondary processors) are now required to manage the risk of *S.* Enteritidis under the *Animal Products Regulations 2021* (Ministry for Primary Industries 2022c).[42] All chicken producers (including those that produce breeder chickens, fertile eggs from breeder chickens, hatcheries that produce day-old chicks, rearer laying chickens, layer chickens and broiler chickens) must have a registered RMP no later than 1 November 2023. Until this occurs, producers must be a listed chicken producer with MPI. The operator of the primary or secondary processing facility is responsible for developing and registering their RMP but the programmes are subject to independent verification.

In relation to *S*. Enteritidis, MPI recommends that documented RMP procedures must:

- set out the procedures the operator will use for identifying, controlling, managing, eliminating, or minimising risk factors;
- describe the steps the operator will take to confirm that the programme is working effectively;
- make provision in relation to tracing and recalling animal material and animal products; provide for appropriate corrective actions (including recalls) to be undertaken where animal material or animal products may be not fit for intended purpose or not in accordance with its labelling or identification; and
- provide for appropriate and auditable documentation, record keeping, and reporting.

Environmental testing for *S*. Enteritidis

As discussed above, routine environmental testing of the poultry production environment was implemented following the detection of *S*. Enteritidis in the New Zealand poultry industry (Ministry for Primary Industries 2022f); prior to this time, no environmental testing was required. Some environmental testing was conducted as part of MPI's investigations into the incursion, and from 6 October 2021 to 5 October 2022, testing for *S*. Enteritidis was conducted under the ECS (Ministry for Primary Industries 2022f).

As discussed in Section 2.2.2, the New Zealand poultry supply relies on a small number of centralised breeders and hatcheries which supply a larger number of rearer operations, which in turn supply a larger number of layer farms. As such, the higher up the supply pyramid that *S*. Enteritidis contamination occurs, the greater the risk for its transmission to more farms and flocks. Therefore, the testing scheme requires the most comprehensive and frequent sampling regime for breeders and hatchery facilities. Testing utilises the most sensitive sample types (particularly, dust and faeces) which maximises the likelihood of detecting *S*. Enteritidis if it is present in a shed or flock. More detail on the sample types and sampling schedules specific to each facility type are detailed in Appendix C.

S. Enteritidis requirements for export markets

Overseas Market Access Requirements (OMARs) were introduced in July 2021 and updated in January 2022 to manage risks posed by *S*. Enteritidis to international trade of poultry and

[42] https://www.legislation.govt.nz/regulation/public/2021/0400/latest/LMS520972.html; accessed 13 January 2023

poultry products (Ministry for Primary Industries 2022e).[43] These include, but are not limited to, day-old chicks, hatching eggs, fresh table eggs and raw egg products.

Source farms must be an export-approved premises (Ministry for Primary Industries 2018a) and there are Official Assurance documentation requirements (Ministry for Primary Industries 2022d). Export regulatory requirements for source farms include a demonstration of *S.* Enteritidis freedom status (Ministry for Primary Industries 2022a, e). The environmental sampling requirements and testing frequency differ from the routine testing of breeder and layer flocks, and are described in Appendix A.4.

Whole Flock Heath Scheme

Under the *Animal Products Notice: Production, Supply and Processing* (Ministry for Primary Industries 2022c), poultry producers most also comply with documented procedures for managing the health of poultry. This must address areas such as disease control, management of agriculture compounds and poultry medicines, measures for feed management and environmental contaminant controls.

Vaccination programme

Vaccination for *Salmonella* is recommended but not mandatory in New Zealand. Vaccination is routinely undertaken in layer and breeder flocks. In a 2016 survey of 28 of the 143 commercial layer farms operating in New Zealand at the time, surveyed flocks from all 28 farms were vaccinated (Kingsbury et al. 2019a).

Currently AviPro Megan® Vac 1 (ACVM registration number A007935) is the only vaccine approved under MPI's Agricultural Compounds and Veterinary Medicines Act 1997 for mitigation of *S*. Enteritidis in New Zealand. Megan® Vac 1 is a live vaccine made from an attenuated strain of *S*. Typhimurium that has mutations in the *cya* and *crp* genes (Curtiss and Kelly 1987, Hassan and Curtiss 1990, Hassan and Curtiss 1994). The New Zealand suppliers (Pacific Vet) report that Megan® Vac 1 is intended for use in broiler, layer and breeder chickens as an immunological aid for the reduction of *S.* Typhimurium, *S.* Enteritidis and *S.* Heidelberg colonisation of the digestive tract and internal organs.[44] It also acts as an immunological aid in the reduction of *S.* Typhimurium and *S.* Enteritidis colonisation of intestinal, visceral and reproductive tract, including egg colonisation. Vaccination involves three doses, which are administered to day-old chicks (via spray administration), and at two and 16 weeks of age (using drinking water administration).

Note that although attenuated, the *S*. Typhimurium Megan®Vac-1 strain has occasionally been isolated from human salmonellosis cases in New Zealand.[45]

[43] https://www.mpi.govt.nz/export/export-requirements/omars/omar-notifications-for-animal-products/; accessed 17 January 2023
[44] https://www.pacificvet.co.nz/products/avipro-megan-vac-1; accessed 21 November 2022
[45] https://surv.esr.cri.nz/PDF_surveillance/ERL/HumSalm/2008/HumSalm2008.pdf; accessed 14 March 2023

Labelling requirements for table eggs

Most packaged eggs and egg products require a label (Ministry for Primary Industries 2018c). Exceptions include eggs sold without packaging, when eggs are packed in front of the buyer, when eggs are sold on the farm where they are laid, and if eggs are sold at a fundraising event (where the funds go to a charity and not the seller).

The required information on labels of retail shell eggs includes:

- The name of the food – eggs.
- A lot identification, for example, a date mark and the premises where the food was packed or prepared.
- The supplier's name and business (street) address in New Zealand. "Supplier" includes the packer, manufacturer or vendor.
- Best before date. MPI currently recommends a shelf life for clean, uncracked eggs of 35 days from the date of lay, regardless of storage temperature.
- Nutrition Information Panel.
- A statement on how eggs should be stored. Note that although there is no legal storage temperature requirement for New Zealand eggs, they have a longer shelf life if kept cool.
- Number of eggs, net weight or volume. This is covered in Weights and Measures Regulations 1999.[46]

Claims about a product being "barn laid" or "free range" are subject to the Fair Trading Act 1986.[47] It is an offence to mislead consumers by incorrectly labelling any product.

4.1.4 Testing programmes and control measures in other countries

International control measures and testing programmes for *Salmonella* in the poultry egg laying industry, including for *S*. Enteritidis, vary widely between countries. Controls and testing programmes have been described in more detail in Appendix C.

Various *Salmonella* serotypes are common in egg layer flocks internationally. In Australia, *S*. Enteritidis had not been detected in layer farms prior to 2018 (Chousalkar et al. 2018b). There was no mandatory programme for *Salmonella* testing of flocks, although a voluntary National *Salmonella* Enteritidis Monitoring Accreditation Program (NSEMAP) was available to all commercial egg farmers. However, regulators responded to an outbreak associated with egg consumption in 2018-19 by undertaking an interstate programme of sampling layer flocks and introducing biosecurity measures to prevent *S*. Enteritidis spread. In response to two separate *S*. Enteritidis outbreaks occurring in 2010 and 2015, the Canadian government introduced monthly *S*. Enteritidis testing programme for egg layer breeders. Environmental testing for *S*. Enteritidis is conducted on both Canadian and US breeder farms as part of a certification scheme. The EU initiated an extended control program for zoonotic diseases, including *Salmonella*, in 2003. Between 2007 and 2010, the United Kingdom (UK) *Salmonella* National Control Programme was implemented according to the requirements of Regulation (EC) No. 2160/2003. Of these programmes, the most comprehensive testing of flocks occurs in the EU

[46] https://www.legislation.govt.nz/regulation/public/1999/0373/latest/DLM301528.html; accessed 8 May 2023
[47] https://www.legislation.govt.nz/act/public/1986/0121/latest/DLM96439.html; accessed 8 May 2023

and UK, with required routine testing of breeder flocks or hatcheries, and layer flocks (as well as broiler flocks) for target *Salmonella* serotypes.

The regulated *Salmonella* serotype/s in flocks also differs by legislative region. *S*. Enteritidis is a target in all regions that monitor flocks. In the EU and UK, *S*. Typhimurium (including monophasic *S.* Typhimurium) is also a target. Furthermore, *S*. Hadar, *S*. Infantis and *S*. Virchow are regulated serotypes in breeder flocks in the EU and UK. A 2019 assessment of priority serotypes for breeding hens in the EU has reported that there is justification for retaining *S*. Enteritidis, *S*. Typhimurium and *S*. Infantis, but that *S*. Virchow and *S*. Hadar could be replaced by *S*. Kentucky and either *S*. Heidelberg, *S*. Thompson or a variable serotype based on national prevalence targets (European Food Safety Authority Panel on Biological Hazards 2019). They also state that a target that incorporates all serotypes is expected to be more effective as the most relevant serotypes in breeding flocks vary between Member States and over time.

The consequences of a positive detection of a target serotype also differs by country. For the North American countries and Europe, detection in layer flocks of *S*. Enteritidis (as well as *S*. Typhimurium in Europe) results in a requirement for all eggs to be diverted for additional treatment, for example pasteurisation, prior to entering the market for consumption. Production from positive flocks is still allowed to occur, but with heightened biosecurity requirements and heightened testing prior to placement of new flocks in sheds that previously housed positive flocks. In the US, a positive flock can be returned to negative following four consecutive batches of 1,000 eggs, tested at two-weekly intervals, testing negative for *S*. Enteritidis. In the UK, a positive test following sampling by the operator places the flock under official control, but this can be overturned if additional sampling by the Competent Authority test negative; and there are different consequences if the only positive detection is from dust, but not faeces/boot swabs. Alternatively, official controls can be removed following negative test results from either caecae and oviducts from 300 birds in the flock, or 4,000 eggs.

The requirement to vaccinate flocks against *Salmonella* differs by region. In the UK, the voluntary industry operated scheme (British Egg Industry Council) Lion Quality requires its members to vaccinate their layer flocks (includes >90% of egg production) (The British Egg Industry Council 2013). Many producers who are not members of the scheme also voluntarily vaccinate their flocks. Under the US FDA Egg Rule, vaccination of layers is voluntary, and states that "*If individual producers have identified vaccines that are effective for their particular farms, we encourage the use of the vaccine as an additional SE prevention measure.*"(United States Food and Drug Administration Department of Health and Human Services 2009). Similarly, *Salmonella* vaccination of layer flocks is recommended in Canada and Australia.

Egg storage temperature requirements also differ by country. The US and Canada require temperature control during transport and storage of eggs; at temperatures of 7˚C (US) and 10˚C or 13˚C (depending on egg grade; Canada). The UK Lion Quality Scheme recommends that eggs are stored at an even temperature and below 20˚C, and that on catering premises and in the home, eggs should be stored in the refrigerator below 8˚C. The EU specifies that eggs should not be refrigerated before sale to the final consumer, but a concrete storage

temperature is not specified. In Australia, it is recommended that eggs are stored chilled, but there is no legislative requirement to do so.

4.1.5 Additional options for risk management

Currently, the only programme looking at prevalence and types of *Salmonella* in New Zealand layer flocks is the on-farm testing programme for *S*. Enteritidis (although some EOL carcasses are tested under the NMD programme which occurs following primary processing). The current on-farm testing programme for *S*. Enteritidis offers the opportunity to provide more information about the prevalence of other *Salmonella* serotypes in breeder and layer flocks and hatcheries; particularly, *S*. Typhimurium which continues to be the most commonly observed serotype clinically in New Zealand. This would not require any additional environmental sampling to be conducted and could be as simple as implementing a multiplex PCR in the testing laboratory for confirming other target serotypes.

Testing for other serotypes would identify farms and sheds with a high flock *Salmonella* prevalence, and consequently, where there is a greater risk for contamination of eggs. It would also indicate the efficacy of interventions implemented to control *Salmonella* generally.

In the event that there is a re-emergence of *S*. Enteritidis in New Zealand layer flocks, egg storage temperature considerations might need to be revisited, particularly if there is evidence that contamination of egg contents has occurred. In particular, options could include requiring refrigeration of eggs at restaurants and food service facilities where large numbers of people have the potential to become sick from contaminated egg product. A further option might be requiring catering facilities to use only pasteurised egg contents in foods containing raw or minimally cooked eggs.

5 EVALUATION OF RISK

5.1 RISK ASSESSMENTS

Key findings

- A 2019 report questioned the validity of the Yolk Mean Time approach to evaluate New Zealand egg shelf life. Due to the absence of *S*. Enteritidis in New Zealand layer flocks at the time, there was considered to be a very low likelihood of egg contents being contaminated with *Salmonella*, and YMT modelling was based on *S*. Enteritidis behaviour in eggs, which may not reflect behaviour by New Zealand poultry-associated serotypes.
- In response to the recent 2021 *S*. Enteritidis DT8, ST11 outbreak, MPI provided a brief assessment of the risk to the poultry industry, New Zealand exports, and human health posed by *S*. Enteritidis in poultry flocks.

5.1.1 New Zealand risk assessments and risk related activities

Quantitative or qualitative risk assessments are structured science-based processes that estimate the probability and severity of illness from consuming food containing biological, chemical or physical contaminants, and are used to guide risk management decisions.

The 2016 Risk Profile (Rivas et al. 2016) assessed that there is little evidence that transmission of *Salmonella* via eggs is a significant transmission route occurring in New Zealand, and that the risk had not changed since the 2011 Risk Profile (Lake et al. 2011). As discussed in that document, MPI published a report considering horizontal transfer and growth of *Salmonella* in chicken eggs, which includes some aspects of a quantitative risk assessment (Ministry for Primary Industries 2015). The report was undertaken to examine the ability of *Salmonella* on eggshells to penetrate the shell and grow during storage, and to determine whether New Zealand retail storage requirements were suitable. The document concluded that on the basis of the information available at the time, it would be prudent to maintain the requirements for handling and storage of eggs that were current at that time. At the time of the document, the New Zealand egg storage requirements were:

- 21 days where the storage/holding temperature may exceed 15˚C,
- 35 days if stored or held at 15˚C or less, or
- Other combinations to be specified, and justified by the producer.

These findings were based on Yolk Mean Time (YMT) calculations. YMT is an arbitrary measure developed for a quantitative process model to assist risk assessment for *Salmonella* in eggs (Whiting et al. 2000). It is based on the storage time at a given temperature whereby the vitelline membrane degrades, allowing *Salmonella* present in the albumen to reach the yolk and grow to levels of concern in 20% of eggs.[48] Thus, it is a measure of the time- and temperature-dependent reduction in intrinsic defences to bacterial growth. In risk assessments it is assumed that no growth is possible before YMT has been exceeded (Thomas et al. 2006). Growth was defined as more than 4 $\log_{10}$ CFU/egg of *S*. Enteritidis from an inoculum of 500 cells into the albumen. The 20% value is arbitrary (initially based on growth in 2/10 eggs) but

[48] Note that other publications have referred to this as “yolk membrane breakdown time” or “yolk mean time”.

allows for differences between individual eggs (Food Standards Australia New Zealand 2009, Ministry for Primary Industries 2015).

Since that time, another report has addressed the question: "Is storage of shell eggs at 15˚C or less for a shelf life of 35 days necessary to protect consumers of New Zealand eggs from salmonellosis?" (Kingsbury and Soboleva 2019a). Findings from that report questioned the validity of the YMT approach to evaluate New Zealand egg shelf life, as follows:

- "Due to the absence of *S*. Enteritidis (which can internally colonise the hen), the main route for *Salmonella* contamination of eggs in New Zealand is via cross-contamination to egg surfaces. Therefore, the effect of storage temperature on survival of *Salmonella* on egg surfaces may be a more important consideration than internal contamination processes. Literature supports that *Salmonella* presence on eggshells does not increase as storage times increase, and most likely declines over time. Thus, the risk from *Salmonella* present on eggshells does not increase by prolonging storage times. Storage temperatures, which delay the loss of viability, will increase the time during which cross-contamination or trans-shell penetration could occur. In some studies, higher storage temperatures (e.g. 25˚C compared with 4˚C or 12˚C) led to a faster reduction in *Salmonella* viability on eggs. However, available data were variable and were deemed insufficient to assess the effect of New Zealand-recommended storage temperatures (≤15˚C and ~20-25˚C) on the survival of New Zealand-relevant serotypes on egg surfaces.
- The YMT model used in the earlier 2015 MPI Risk Assessment was based on inoculation of egg albumen with high numbers (500 CFU) of *S*. Enteritidis, a serotype that has not been identified in the New Zealand egg layer environment. Based on results from international studies, some non-Enteritidis, New Zealand-relevant serotypes are able to internalise and survive in egg albumen. However, data were considered insufficient to assess the effect of New Zealand-relevant storage temperatures on the ability of New Zealand-relevant serotypes to internalise eggs, survive in albumen, and/or grow in egg yolk."

Recommendations from that study guided the study by Kingsbury et al. (2019b) that demonstrated; i) reduced survival of New Zealand–relevant *Salmonella* isolates on eggshells stored at 22˚C compared with 15˚C, (ii) a decline of survival of *Salmonella* at both temperatures over time, and (iii) an absence of detection of *Salmonella* in egg contents at any storage time or condition. The 2019 assessment,[49] together with this experimental evidence, were two factors that supported the 2018 change in New Zealand egg storage duration to 35 days regardless of storage temperature. However, these assessments were made before *S*. Enteritidis was detected in New Zealand poultry flocks.

Although not a quantitative risk assessment, MPI have also conducted a rapid assessment of the risk posed by *S.* Enteritidis in poultry flocks in response to the 2021 *S.* Enteritidis outbreak (Ministry for Primary Industries 2022i). *S*. Enteritidis is not new to New Zealand, however it is new in poultry. It presents risks to human and animal health, and international trade, and will continue to be a problem without consistent and enforceable controls. Key points were:

[49] Note that the work conducted for this assessment was completed in 2018, but the report was not published until 2019.

- Extent of *S*. Enteritidis spread in poultry flocks: A delimiting survey to determine the extent and prevalence of *S*. Enteritidis was conducted on the 25 largest egg-laying operations (together supplying 80% of the eggs produced domestically). *S.* Enteritidis was not detected on any additional farms during the survey. Further testing of both egg layer and broiler operations was subsequently required through an Emergency Control Scheme (ECS; see section 4.1.1). A low prevalence of *S*. Enteritidis was found (0.3% positive samples; the number of samples tested and positive farms were not reported). The report was published before the ECS moved to routine sampling under the regulatory framework, so data from that testing was not available.
- The point of contamination in the supply chain affects the risk of dissemination and to human health. The report noted that there had been a lack of microbiological controls in these operations under the *Animal Products Act 1999* (see Section 4.1).
- An *S*. Enteritidis outbreak could be detrimental to public health because chicken meat and eggs are staple food items for many New Zealand households.
- The higher hospitalisation rate compared with all salmonellosis cases suggested the outbreak *S*. Enteritidis strain caused more severe illness, although this may be a detection bias.
- There was a risk to exports because any export of *S*. Enteritidis could affect New Zealand's trade reputation as a responsible exporter of high-quality product and export market access.
- Without preventative monitoring, surveillance, and prompt 'detection' action controls, there was a risk of spread of *S.* Enteritidis because the source of the *S.* Enteritidis in commercial chicken flocks had not been identified.

Risk assessments relating to egg safety for other countries are summarised in Appendix B.2.4.

5.2 EVALUATION OF RISK FOR NEW ZEALAND

Key findings

- **RMQ1:** Detection of the *S*. Enteritidis DT8, ST11 strain SE_2019_C_01 in layer flocks has the potential to increase the risk to the New Zealand layer industry and to consumers of eggs. The potential for transovarian transmission of *S*. Enteritidis to eggs via the breeder flocks at the apex of the supply chain could result in widespread dissemination through the layer poultry supply chain. Colonisation of layer flocks by *S*. Enteritidis poses a greater risk for consumers because egg contents are more likely to be contaminated than occurs via flock colonisation by other *Salmonella* serotypes. The outbreak caused by this strain was the only foodborne salmonellosis outbreak over the period assessed in this Risk Profile where there was strong evidence for eggs as a vehicle (although poultry meat was also considered a potential source.). There is some evidence that this strain poses a greater risk to human health than other *Salmonella* serotypes because it has a higher hospitalisation rate. There is also a risk to international trade of hatching and table eggs.
- The residual level of risk will be determined by the efficacy of the new control measures implemented to detect flock colonisation, eliminate colonised flocks, and control any dissemination of *S*. Enteritidis. At the time of this report, the *S*. Enteritidis SE_2019_C_01 strain has only been detected in a limited number of layer flock, broiler and breeder farm environments, and there have been no human cases infected with the strain since

February 2023. Furthermore, the emergence of this strain has not had a material impact on overall salmonellosis case numbers. Although this strain has the potential to increase the risk to consumers of eggs, information to date suggests that risk management procedures have been effective at controlling the risk.

- The risk associated with non-Enteritidis *Salmonella* serotypes in and on eggs does not appear to have changed since the 2016 Risk Profile. This conclusion is based on a low prevalence of non-Enteritidis serotypes in New Zealand layer flocks in a 2016 survey, the static incidence of salmonellosis, and few outbreaks involving non-Enteritidis serotypes where eggs were suspected. Detection of non-Enteritidis serotypes from egg contact surfaces in New Zealand packhouses show that eggs can potentially be contaminated by non-Enteritidis serotypes. However, experimental evidence showed *Salmonella* numbers reduce with time on clean eggshells at New Zealand-relevant storage temperatures and trans-shell transmission into egg contents was not detected.
- Important knowledge gaps include:
  - Current prevalence data for non-Enteritidis *Salmonella* in layer poultry breeders, hatcheries, and layer flocks; and how the increasing proportions of hens housed in cage-free systems are influencing prevalence.
  - A lack of evidence whether the *S*. Enteritidis SE_2019_C_01 strain is indeed capable of transovarian transmission, and the behaviour of this strain in and on eggs at New Zealand-relevant storage temperatures.
  - Whether *S*. Enteritidis SE_2019_C_01 is disseminated in the wider environment and if there are unknown reservoirs (such as backyard poultry).
  - The route by which *S*. Enteritidis SE_2019_C_01 was introduced into the New Zealand poultry industry.
  - Lack of recent national nutrition surveys to assess poultry consumption trends and apportion consumption data for at risk demographics.
- **RMQ2:** The most effective overall strategy to control *Salmonella* in and on eggs is by applying multiple interventions throughout the egg production chain to control colonisation of layer chickens and prevent contamination of the farm environment. Environmental management includes controlling the food and water supply, biosecurity and pest management, and ensuring effective cleaning regimes are in place. Vaccination is widely practiced on New Zealand layer farms, and can reduce, but not prevent, flock colonisation, shedding, and contamination of eggs. The addition of prebiotics, probiotics, bacteriophages, organic acids and/or phytochemicals to feed provides some protection against *Salmonella*.
- Post-harvest control measures include optional egg washing, which may reduce *Salmonella* numbers on egg surfaces but might promote trans-shell transmission if performed incorrectly. UV treatment may also reduce *Salmonella* numbers on egg surfaces and is used in some New Zealand packhouses. Pasteurisation or fully cooking eggs inactivates *Salmonella* in egg contents. Other effective hazard mitigation behaviours for consumers include discarding eggs which are dirty, cracked or past their use-by date, and washing hands and surfaces following contact with raw eggs. Maintaining refrigeration of eggs post-lay will control the growth of any *Salmonella* that might be present in the egg contents.

- **RMQ3:** The current shelf life for New Zealand eggs is 35 days regardless of storage temperature. Experimental studies show that *Salmonella* present on clean eggshells will not grow, and will die faster at warmer storage temperatures (for example, room temperature compared with refrigeration). However, warmer temperatures promote faster breakdown of the vitelline membrane and more rapid growth of any *Salmonella* present in egg yolk, whereas *Salmonella* will not grow at refrigeration temperatures. New Zealand shelf life considerations were guided by the very low likelihood that *Salmonella* would be present in egg contents, but the risk for contamination of egg contents is higher for *S*. Enteritidis because it is potentially capable of transovarian contamination of eggs. Data suggest that the current risk management interventions are effectively mitigating *S*. Enteritidis in New Zealand layer flocks. However, a reconsideration of shelf life guidelines would be important if the strain were to re-emerge and become endemic in New Zealand layer flocks. A knowledge of whether *S*. Enteritidis SE_2019_C_01 is capable of transovarian transmission, and the behaviour of this strain in and on eggs at New Zealand-relevant storage temperatures and refrigeration would inform modelling for shelf life considerations.
- **RMQ4:** The best approach to gather information on the prevalence of *Salmonella* on New Zealand eggs is by environmental sampling at layer farms. Effective sampling programmes include both faeces and dust, maximise the number of samples taken, and conduct sampling during periods when the flock are more likely to be stressed and shedding *Salmonella*, such as at the onset of lay. Testing egg contact surfaces at packhouses can also indicate if contamination of egg surfaces is occurring.
- The purpose of the newly implemented testing programme for *S*. Enteritidis in New Zealand breeder, layer and broiler flocks and hatcheries is to maximise the likelihood of *S*. Enteritidis detection, followed by mitigation if detected to ensure that eggs that might be contaminated by *S*. Enteritidis will not reach the consumer. The testing programme appears as rigorous as that conducted in the EU with respect to sampling frequency, timing, and sensitivity of sample types. However, the EU programme also regulates *S*. Typhimurium in all flocks and monitors three other serotypes in breeder flocks.
- Testing regulatory framework samples for total *Salmonella* prevalence, and targeting other serotypes of higher concern such as *S*. Typhimurium in addition to *S*. Enteritidis, would provide valuable information on the exposure of New Zealand eggs to other *Salmonella* serotypes.

5.2.1 RMQ1: Considering the detection of *Salmonella* Enteritidis in chicken hatcheries/day-one chicken suppliers in 2021, how has the public health risk from *Salmonella* in or on eggs changed since the 2016 Risk Profile update?

The key finding from the 2016 Risk Profile (Rivas et al. 2016) was that based on the reduced incidence of salmonellosis and few events where epidemiological evidence linked eggs with salmonellosis, the public health risk from *Salmonella* in or on eggs consumed in New Zealand had not changed since an earlier 2011 Risk Profile (Lake et al. 2011). Whole, fresh eggs sold in New Zealand could be contaminated with *Salmonella*, but this was contributing to only a minor (but undefined) proportion of human illness. *Salmonella* was detected on (but not inside) New Zealand eggs at retail in earlier studies (Wilson 2007).

To address RMQ1, both the overall risk of salmonellosis from eggs and the specific risk of salmonellosis from *S.* Enteritidis need to be considered.

Regarding the overall risk of salmonellosis, there is no strong evidence to suggest the risk from non-Enteritidis serotypes has changed, although there are important data gaps and these reveal uncertainty. New information comes from a layer farm survey, widespread changes in poultry housing, signals of changing consumer practices and recent human health data.

The NMD programme tests some EOL birds and during the period 2016-2022, *Salmonella* was detected on 0.14% of 1,476 EOL carcasses. While this suggests a low prevalence, primary processing practices are designed to reduce the microbial numbers (including pathogens) on carcasses and the actual number of birds tested was very low compared to the national flock of live layers. Since the 2016 Risk Profile, some new data on the prevalence of *Salmonella* in New Zealand layer farms has become available. Data from the 2016 cross-sectional survey of 67 layer sheds on 28 New Zealand layer farms found that the highest prevalence, based on dust sampling, was 28%, although *S.* Enteritidis was not detected in any samples. However, some farms had a high prevalence of *Salmonella*-positive laying shed environmental samples, and genetically related isolates were also detected on egg contact surfaces from the egg packhouse of those farms. Once contaminated, these surfaces would be a source of external contamination to eggs subsequently processed on the same surfaces. While these data do not inform on the risk of *S.* Enteritidis specifically, they do indicate the potential for contamination on the outside of the egg at layer farms, especially on farms with higher *Salmonella* prevalence. Further information to inform on risk could be gathered by targeted egg surveys from layer farms with high *Salmonella* flock prevalence.

Since the 2016 Risk Profile, there has been a major shift in the proportions of different poultry housing systems. The proportion of the national flock housed in conventional cage systems has reduced from the majority to a small minority, with greater proportions being housed in colony cages, barn or free range systems. As of December 2022, 10% of the national layer flock were housed in conventional cages, 33% in colony cages, 24% in barn and 33% free range. The increase in cage-free systems has the potential to impact on the *Salmonella* prevalence in laying flocks. However, there is currently no scientific consensus regarding the impact that different housing systems have on *Salmonella* contamination of eggs. This is due to the complexity of confounding factors and variables such as flock size, flock density, single versus multi-age, biosecurity and weather effects. The balance of evidence, including the 2019 New Zealand study (Kingsbury et al. 2019a), supports a lower occurrence of *Salmonella* among poultry kept in cage-free systems. Furthermore, the New Zealand cross-sectional survey found *Salmonella* prevalence in cage-free flocks was significantly lower than in caged flocks. Assuming that data is representative of New Zealand flocks in general, the increase in cage-free systems might be expected to result in an overall reduction in the prevalence of *Salmonella* in layer flocks.

Prevalence on its own is not a complete indicator of food safety, because the salmonellosis risk is also affected by the volume of the food type consumed, *Salmonella* numbers present, serotype virulence, and consumer practices. The volume of eggs produced and available to New Zealand consumers has fluctuated since the 2016 Risk Profile was produced. Recent

egg consumption data are not available for New Zealand although poultry data indicate that whole, raw eggs are still the main product being prepared for eating by consumers rather than pre-separated and/or pasteurised egg contents. International studies have reported an increasing popularity of home-made foods containing raw eggs, which potentially increases the risk of salmonellosis. Australian reports have documented that salmonellosis outbreaks due to raw and undercooked eggs are increasing (OzFoodNet Working Group 2015, Whiley and Ross 2015). New Zealand consumer-level studies of egg consumption are needed to understand whether there have been changes to both overall consumption (serving sizes, serving frequencies) and the form of the eggs being consumed (particularly considering raw egg consumption).

The yearly incidence of notified salmonellosis in New Zealand was relatively static during the period covered by this Risk Profile (from 2015 to 2021, Table 6, Figure 6), noting that the lower notification rates for 2020 and 2021 could be attributed to the impact of the COVID-19 pandemic public health response. The notification rates for salmonellosis from 2015 to 2019 suggest that the overall risk from *Salmonella* has not increased since the previous 2011 Risk Profile. However, the number of hospitalised cases appears to be higher in 2018, 2020 and 2021 compared to other years, signalling that there might be a change in severity of disease (although note that the proportions of salmonellosis hospitalisations relative to case notifications was during 2020 and 2021 because there was a reduction in notifications due to aspects of the COVID-19 public health response). It might become important to investigate this further, such as identifying any associations between severe disease, specific *Salmonella* types and exposure risk factors (including whether domestic foods were serving as vehicles of infection). Antimicrobial resistance among non-typhoidal *Salmonella* isolated from New Zealand human, animal and environmental samples remains relatively low.

Of the 13 salmonellosis outbreaks since 2015 where eggs were the suspected or implicated vehicle of infection, there was a single outbreak with strong evidence, which was the *S*. Enteritidis 2021 outbreak, although poultry meat was also considered a potential source. Egg-containing foods were implicated by strong evidence in four of the 204 salmonellosis outbreaks between 2000 and 2009. Between 2010 and 2014, eggs were implicated in three of the 106 reported salmonellosis outbreaks; there was strong evidence in two of these. Considered together with outbreaks identified in the earlier Risk Profiles, chicken eggs do not appear to be an important contributor to salmonellosis caused by non-Enteritidis serotypes in New Zealand.

Regarding the specific risk of salmonellosis from *S.* Enteritidis, there is evidence to show that the appearance of this serotype in New Zealand poultry has increased the risk of salmonellosis from eggs. However, the level of risk is strongly connected to the effectiveness of controls.

Following the detection of *S*. Enteritidis in poultry in 2021, a delimiting survey to determine the extent and prevalence of *S*. Enteritidis was conducted in 25 of the largest egg-laying operations (together supplying 80% of the eggs produced domestically). *S*. Enteritidis was not detected on any additional farms during the survey. Subsequent testing of poultry flocks for *S*. Enteritidis as part of the ECS only found this serotype on a few farms, some of which are no longer operating. Furthermore, there have been no detections of *S*. Enteritidis from any New

Zealand rearer or layer shed environments since October 2021. These data suggest that, currently, *S*. Enteritidis is not widespread nor established in the New Zealand layer industry.

However, the detection of *S*. Enteritidis DT8, ST11 strain SE_2019_C_01 in layer poultry flocks and hatcheries has increased the potential risk of salmonellosis from the consumption of eggs for the following reasons:

- *S*. Enteritidis is more invasive in chickens than other serotypes. Phage type DT8 strains are potentially capable of transovarian transmission to unlaid eggs. This poses a risk for breeder flocks due to the potential for transmission to chicks. Given the placement of breeders at the apex of the supply chain, there is greater potential for amplification through supply chain. Chicks in their first days of life are highly susceptible to colonisation by *Salmonella*; hence both externally and internally contaminated eggs also pose a risk via cross-contamination to neighbouring newly hatched chicks.
- Importantly, if transovarian transmission occurs in table eggs destined for human consumption, any contamination of the yolk provides an opportunity for the *S*. Enteritidis to grow to high numbers, depending on the storage temperature. These numbers are higher than can be found on the external egg surfaces. This poses a significant risk of host infection following consumption of eggs if they are consumed raw or undercooked. Cross-contamination through handling eggs in the kitchen also poses a wider risk of foodborne salmonellosis.
- *S*. Enteritidis contributes to a significant proportion of egg-associated salmonellosis in the EU and North America. There is good evidence linking the presence of *S*. Enteritidis SE_2019_C_01 in the New Zealand poultry industry with human salmonellosis cases during the 2021 outbreak.
- There is evidence that *S*. Enteritidis SE_2019_C_01 poses a greater risk to human health than other *Salmonella* serotypes or even other strains of *S*. Enteritidis. In New Zealand. cases infected with this strain were more likely to be hospitalised. Over a similar reporting period, the hospitalisation rate for cases infected with *S*. Enteritidis SE_2019_C_01 was higher than that of all cases of salmonellosis, and all cases infected with *S*. Enteritidis (which includes other *S*. Enteritidis strains). However, it remains possible that this is due to case ascertainment bias.
- The outbreak due to *S*. Enteritidis SE_2019_C_01 was the only New Zealand outbreak since 2015 where there was strong support for eggs as the vehicle for human salmonellosis. Total case numbers were also higher than for other potentially foodborne outbreaks of salmonellosis over the same period. Despite this, the outbreak was not considered to materially affect the total number of salmonellosis cases reported for 2021. This might be because the strain was not widely disseminated in the poultry industry.

The residual risk posed by *S*. Enteritidis depends on the efficacy of control measures. New regulatory requirements include an RMP for all sectors of the industry, microbiological testing of the poultry environment for *S*. Enteritidis, procedures for the tracing and elimination of *S*. Enteritidis from the poultry supply chain where detected, and changes to Overseas Market Access Requirements. New Zealand testing requirements for *S*. Enteritidis in the poultry production environment were deemed to be at least as stringent as those used internationally with respect to sample types, numbers, frequency and timing of testing.

Despite the above, *S*. Enteritidis colonisation of flocks could be undetected due to a low flock prevalence or if flock colonisation occurred post-testing. Effective post-lay egg controls would be important to protect consumers.

Data gaps and options to address gaps

***Salmonella* prevalence in layer flocks and eggs:** The main source of prevalence data for *Salmonella* in layer flocks is the 2016 cross-sectional survey of 28 layer flocks, but this was from a single point in time. There are no recent data on the prevalence and serotypes of non-Enteritidis *Salmonella* occurring during primary production of eggs. Options to address these gaps might include collating prevalence data for all *Salmonella* isolated during routine *S*. Enteritidis sampling of breeder and layer flocks, as well as hatcheries. This might also involve testing for additional priority serotypes such as *S.* Typhimurium. Such data could indicate the efficacy of regulatory control changes on *Salmonella* ecology in flocks, and the effect that the change in housing systems is having on *Salmonella* prevalence. Farms identified as having a high prevalence of *Salmonella* could be offered guidance for implementing additional control measures. To better understand the risk to consumers posed by *Salmonella*-positive layer flocks (see below), these farms could also be used for the testing of eggs for the presence of *Salmonella*.

Egg consumption data: The egg industry produces data on the yearly production of eggs in New Zealand. However, there have been no recent nutritional surveys conducted. Available data are now 14 years old (the Adult Nutrition Survey) or 21 years old (the Childrens' Nutrition Survey). Such surveys also collect demographic data, which enables investigators to assess whether exposures differ between population groups. Changes in consumer food preferences, consumption amounts, and the emergence of new foods have likely occurred in the since the surveys were undertaken.

Egg survival dynamics, transovarian and virulence potential of *S*. Enteritidis SE_2019_C_01: The potential for transovarian transmission of the *S*. Enteritidis SE_2019_C_01 outbreak strain is not known, although it has the same phage type and sequence type of other *S*. Enteritidis strains shown to be capable of transovarian transmission and/or causing large outbreaks associated with eggs. There are also no data on whether this strain is more transmissible or invasive within poultry flocks. Only very minimal testing of egg contents and post-mortem analysis of colonised flocks have been conducted. Even for strains that are capable of transovarian transmission to eggs, the prevalence of egg contamination via this route may be very low in naturally contaminated flocks, which would require the testing of a large number of eggs (reviewed by Gantois et al. 2009). Egg contamination rates have been reported to be higher from chickens that have been artificially inoculated with *Salmonella* (up to at least 20% prevalence in some studies, depending on the experimental infection conditions).

A better understanding of the transovarian potential of this strain could also involve testing eggs and reproductive tissue from colonised layer or breeder chickens. A limitation of this approach is that based on current regulations, layer flocks determined to be positive for this strain will likely be depopulated (although farmers instead opt for treatment of eggs with a validated method where *S*. Enteritidis is reduced to appropriated levels). Maintaining an *S*.

Enteritidis-positive flock for research purposes presents a significant cost and biosecurity risk. Given this restriction, any egg sampling would need to occur rapidly and would only represent the situation at the time of flock depopulation. Another option could be using experimentally inoculated flocks housed in a strict biosecurity containment environment. Alternatively, in vitro applications such as "organ-on-a-chip" could be used to compare the invasion and virulence potential in different poultry cell lines. The *S*. Enteritidis SE_2019_C_01 strain, non-Enteritidis *Salmonella* isolates from poultry, and other *S*. Enteritidis strains endemic in New Zealand that have not been detected in poultry, could be compared with this method. "Organ-on-a-chip" technology involves a microfluidic device containing a cell type of interest in close recapitulation of the original tissue structure, function and physiology (i.e. a three-dimensional system) that has previously been used to investigate microbial invasion into different tissue types (Puschhof et al. 2021).[50] To our knowledge, this approach has not been used to examine transovarian potential of *S.* Enteritidis, but other studies have examined *S*. Enteritidis invasion in two-dimensional chicken reproductive system cell lines such as an ovarian follicle line (Dawoud et al. 2011).

The genetic determinants for transovarian transmission are still not well understood. However, genomic analysis of the SE_2019_C_01 strain has the potential to provide insights into alleles that might contribute to both poultry invasiveness and human pathogenesis.

As discussed in Section 2.4.4, there is evidence that *S*. Enteritidis survives better in egg albumen than other serotypes. A further option could be to assess the ability of the *S*. Enteritidis SE_2019_C_01 strain to survive on egg surfaces, in albumen, and grow in yolk at New Zealand-relevant storage temperatures. Methods used by Kingsbury et al. (2019a) would be applicable.

Extent of *S*. Enteritidis SE_2019_C_01 dissemination in the wider environment: Testing for *S*. Enteritidis is limited to the poultry housing environment, although the SE_2019_C_01 strain has also been isolated from wild animals such as rodents and a hedgehog. These detections raise the possibility that the strain could have spread outside of the poultry environment, but whether this has occurred, and if so, to what extent, is not known. Possible options to explore this could involve testing rodents or hedgehogs in areas near poultry establishments on which *S*. Enteritidis had previously been detected. Alternatively, the strain might spuriously be detected in other environmental surveys.

Risk to poultry of other *S*. Enteritidis strains endemic in New Zealand: There is no information on the risk to poultry of other *S*. Enteritidis strains endemic within other non-poultry sources in New Zealand. There is no evidence for these occurring in New Zealand poultry; however, if this were to occur, they should be detected through the newly implemented routine testing for *S*. Enteritidis in New Zealand poultry flocks.

Occurrence of *S*. Enteritidis SE_2019_C_01 in non-commercial poultry and backyard flocks: Newly implemented regulatory measures to control *S*. Enteritidis target commercial chicken operations only; they do not target non-chicken poultry such as ducks and turkeys

[50] Organ-on-a-chip technology is available and under trial at ESR for other applications.

(although these are limited numbers of these) or backyard poultry flocks. There are also no data on the occurrence of *S*. Enteritidis in backyard flocks.

5.2.2 RMQ2: What interventions are available to manage the risk from *Salmonella* in and on eggs and what is known about their effectiveness?

The most effective overall strategy to control *Salmonella* in and on eggs is by applying multiple interventions throughout the egg production chain to control colonisation of chickens and prevent contamination of the farm environment. As discussed in Section 2.3.3, a *Salmonella* contamination point earlier in the production chain (breeders, followed by hatcheries) poses the greatest risk of dissemination through the supply chain. As such, breeder flocks must be obtained from *Salmonella*-free grandparent chicks reared in quarantine facilities. Stringent measures are critical, and include strict biosecurity control with regards to personnel and visitors, flock movement, and more intensive environmental testing for *S*. Enteritidis.

On-farm risk factors for colonisation of chickens are detailed in Section 2.3.3 and Appendix A.3, and elements of pre-harvest controls are also addressed. Key controls consist of the following:

- **Strict biosecurity measures:** Examples of measures are discussed above. The detection of the *S*. Enteritidis SE_2019_C_01 outbreak strain from environmental sources, especially rodents, means that *S*. Enteritidis (and other serotypes) can be transferred through farms (shed-to-shed, or range-to-range). This places a strong emphasis on controlling rodent populations, reducing potential rodent harbourage sites such as long grass, and eliminating roosting sites and access by any wildlife to layer sheds and other farm buildings (including structures that hold feed, service personnel, store equipment, etc.). Because of the extended length of time that *Salmonella* can survive in dry conditions such as dust, there is potential for transmission between consecutive flocks in layer shed. This can be addressed by thorough cleaning and disinfection of layer houses after each depopulation.
- **Vaccination:** Vaccination is the most commonly used serotype-specific risk reduction practice for layer hens (reviewed by (Gast et al. 2022)). It aims to reduce, but seldom prevents, hen susceptibility to infection, vertical and horizontal transmission of infection, poultry house environmental contamination, and the incidence of egg contamination. Vaccine efficacy is improved by multiple booster doses. However, efficacy is reduced if the hen consumes high pathogen numbers or if hen's immunity has been suppressed and as the birds age. Commercially available vaccines may contain inactivated (killed) or attenuated (live) *Salmonella*. As discussed in Section 4.1.3, most layer flocks are vaccinated with the attenuated *S*. Typhimurium vaccine AviPro Megan® Vac 1, which offers some protection against *S*. Typhimurium, *S*. Enteritidis and *S*. Heidelberg.
- **Feed additives**: More recently, a number of feed additives have become available that are designed to reduce or prevent gastrointestinal colonisation by *Salmonella*. Data on the efficacy of feed additives has been assessed in a recent review (Gast et al. 2022). Additives may include the following, which are often used in combination:
    - Probiotics: direct-fed microbes for competitive exclusion to impede *Salmonella* colonisation;

- Prebiotics: compounds that are utilisable by beneficial gut microbiota, and promote their growth;
- Phytochemicals: plant-derived antimicrobials such as essential oils, botanicals, herbs, and oleoresins;
- Organic acids: added to feed to inhibit the growth of fungi and to limit the growth and survival of pathogens such as *Salmonella*. Examples include short-chain fatty acids, propionate, formate and butyrate;
- Bacteriophages: viruses that specifically target bacteria as a host. The high level of specificity of phages (often, specific to only a few serotypes or strains within a serotype) means that they are typically prepared as a phage mixture when intended for therapy. Development of resistance and changing populations of *Salmonella* can limit the efficacy of phage therapy.

- *Salmonella* surveillance is valuable to detect colonisation of flocks, and to direct mitigation efforts where issues are detected. Depending on the serotype detected and regulatory requirements, mitigations may include depopulation of flocks where target serotypes are detected or channelling raw eggs for pasteurisation/heat treatment. They may also include more stringent cleaning and disinfection practices, a review of biosecurity, and more intensive surveillance to follow the efficacy of interventions.

Post-harvest *Salmonella* control measures are described in Section 2.4. Important measures include candling and sorting of eggs so that eggs reaching retail are clean and do not contain cracks that might permit entry of *Salmonella*, noting that this process does not detect microcracks nor any damage occurring after packaging. Egg washing can also reduce *Salmonella* numbers on egg surfaces, although the degree of efficacy depends on the methodology and sanitisers used. Egg washing may also promote *Salmonella* internalisation of eggs if performed incorrectly. UV treatment of eggs is conducted in some New Zealand packhouses. This has the potential to reduce the numbers of viable *Salmonella* on eggshells, but not within eggshell pores or egg contents. However, effectiveness of this practice under the parameters used by New Zealand packhouses is not known. Pasteurisation is the most effective method for eliminating *Salmonella* from egg contents during processing. Irradiation of eggs is also effective, but affects sensorial and functional properties of the egg, limiting its utility.

The most important control measure in the domestic setting for managing the risk from *Salmonella* in and on eggs is by properly cooking eggs and not consuming them raw or undercooked. A generic flowsheet has been produced for Critical Consumer Handling of eggs and egg products, developed in the frame of the SafeConsumE project which aims to reduce the burden of foodborne disease in Europe (Cardoso et al. 2021, Junqueira et al. 2022).[51] The flowchart lists steps from egg acquisition by the consumer, down to cooking of leftovers, and indicates to the consumer actions or choices that can significantly reduce, eliminate, or prevent the hazard. Steps where actions can influence hazard mitigation include:

- Egg acquisition: egg selection criteria and safety checking methods; give priority to “best before” dates and check the cartons for cracked or dirty eggs.

[51] https://safeconsume.eu/; accessed 22 May 2023

- Handling whole eggs: consumers are advised not to wash dirty eggs at home before storage, but washing dirty eggs before food preparation has been recommended in some places. Hand washing following handling of eggs.
- Egg storage: considerations for storage temperature, duration and storage method.
- Cooking or preparation without cooking: considerations for cooking method, cooking time, doneness checking methods, recipes using raw/undercooked eggs.
- Cooling and storing leftovers: considerations for likeliness of producing egg leftovers, leftover storage temperature, and storage time.
- Cooking leftovers: as listed above for cooking.

Presuming the current shift in consumers' preference and increasing desire for raw food products reported in Australia is also true for New Zealand, more informed guidelines are required regarding the preparation of foods containing raw eggs. Australian health and safety regulators recommend that raw egg based foods should be prepared with only fresh, properly handled eggs, acidified to pH ≤4.2, and stored at 5˚C for no more than 24 hours (New South Wales Food Authority 2023).

5.2.3 RMQ3: What information is available to advise industry regarding shelf life and storage conditions for eggs in relation to the risk from *Salmonella*?

As discussed in Section 4.1.4, egg storage temperature requirements differ by country. These are in part guided by whether *S*. Enteritidis is endemic in layer flocks, because this poses a higher risk for contamination of egg contents via transovarian transmission.

As discussed in Section 2.4, storage temperature affects the following aspects of *Salmonella* behaviour in and on eggs:

- **Survival on eggshells:** *Salmonella* (including New Zealand egg-associated isolates) will not grow on visibly clean eggs and the number of viable *Salmonella* will decrease over time. The rate of decrease is affected by storage temperature. A New Zealand study found that egg-associated *Salmonella* isolates survived better on eggs at 15˚C compared with 22˚C. Other data indicates that survival is even better at refrigeration temperatures.
- **Trans-shell transmission:** *Salmonella* (all serotypes) have the potential to penetrate eggshells and enter into egg contents. Experiments indicate that lower temperatures may reduce the proportion of eggs where *Salmonella* internalisation has occurred, but cooler storage temperatures will not prevent this from occurring. New Zealand isolates were not observed to internalise into egg contents at storage temperatures of either 15˚C or 22˚C. Experiments were conducted on clean, uncracked eggs. As mentioned above, microcracks can be present plus egg damage might occur along the transport chain, which could facilitate contamination of egg contents. There are no data on the proportion of unclean or cracked eggs present at retail.
- Older studies have suggested that condensation on the eggs, which might occur during cold chain distribution, may increase *Salmonella* penetration of the shell. However, recent studies have found little evidence for this occurring and suggested that this risk has been overstated.
- **Survival in albumen:** Any *Salmonella* present in the albumen will not grow in the absence of contamination from the yolk. *S*. Enteritidis might survive better in albumen than other serotypes. Some studies reported that viability declined at 4˚C.

- **Vitelline membrane degradation:** The vitelline membrane separating the albumen from the yolk breaks down faster at higher temperatures, allowing *Salmonella* access to the nutrient-rich yolk.
- **Growth in yolk:** *Salmonella* (all serotypes) will grow in the nutrient-rich yolk. Growth is inhibited at 4˚C, but growth rate increases with increasing storage temperature.

Together, the above indicates that cooler temperatures prolong survival of *Salmonella* present on the outside of eggs but reduce the risks from *Salmonella* present inside whole eggs, either as a result of trans-shell or transovarian transmission.

Prior to 2018, New Zealand eggs had a shelf life of 35 days if held at 15˚C or less, or 21 days where the storage temperature may exceed 15˚C. This had been guided by YMT modelling based on the storage time at a given temperature whereby the vitelline membrane degrades, allowing *Salmonella* present in the albumen to reach the yolk and grow to levels of concern. However, the egg shelf life was changed in 2018 to 35 days regardless of storage temperature. The change was in part guided by:

- The YMT was likely not relevant to New Zealand egg storage considerations because there was a very low likelihood that contents of New Zealand eggs were contaminated by *Salmonella*. This conclusion was based on:
    - experimental evidence that showed a lack of egg internalisation by New Zealand isolates;
    - absence of *S.* Enteritidis in New Zealand poultry at the time; and
    - absence of detection of *Salmonella* from the contents of New Zealand eggs in earlier studies of retail eggs.
- The lack of epidemiological evidence that New Zealand eggs were an important pathway for salmonellosis.
- The experiments showing that storage at 15˚C was not more protective than 22˚C with respect to *Salmonella* survival on eggshells.

Detection of *S*. Enteritidis in New Zealand layer flocks raises the question of whether egg storage temperatures should be reconsidered. Although the outbreak of *S*. Enteritidis was associated with eggs (as well as poultry meat), the available evidence at the time of this current Risk Profile indicated that the risk management mitigations in place were successfully managing the *S.* Enteritidis risk. *S*. Enteritidis was not detected in New Zealand eggs (although minimal testing has been conducted), there were no detections of *S.* Enteritidis from rearer or layer farms since October 2021, and there were no recent (since February 2023) salmonellosis cases caused by the outbreak strain where there was strong evidence for eggs as the source of infection.

However, in the event that egg-associated cases in New Zealand increase as a result of infection by *S*. Enteritidis SE_2019_C_01 or another *S*. Enteritidis strain, or if *S*. Enteritidis becomes endemic in New Zealand poultry, it would be pertinent to reconsider egg storage temperatures. Should this occur, it would be beneficial to have improved information on the behaviour of the *S*. Enteritidis SE_2019_C_01 outbreak strain in and on eggs to guide risk models. Relevant data gaps include:

- Is this strain better able to penetrate eggshells and enter into egg contents than other New Zealand egg-associated strains, or other *S.* Enteritidis strains?
- How well does this strain survive in albumen at New Zealand-relevant storage temperatures?
- How well does this strain grow in yolk at New Zealand-relevant storage temperatures?
- Is this strain capable of transovarian transmission to eggs?
- What proportion of eggs at retail are not visibly clean or contain cracks, and as such are more likely to permit trans-shell transmission?

As discussed in Section 4.1.4, the EU requires that eggs should not be refrigerated before sale to the final consumer. This requirement is despite *S*. Enteritidis being endemic in European layer flocks and causing a significant amount of salmonellosis attributed to eggs. A recent assessment has concluded that these requirements are based on an over-emphasis on *Salmonella*-related risks due to condensation forming on the eggshells (Fikiin et al. 2020). However, they underestimate other more substantive hazards for egg safety and quality, particularly the risk for *S*. Enteritidis to grow in egg contents. Furthermore, as discussed in Appendix B.2.4, recent modelling from the US, where *S*. Enteritidis is also endemic in layer poultry supports refrigeration of eggs where *S*. Enteritidis contamination is possible. The model estimated that for egg contents contaminated with *S*. Enteritidis, storage for 5 days at 18˚C instead of 7˚C would result in 30-fold higher numbers of *S*. Enteritidis, and a 47-fold increase in salmonellosis risk from consumption of those eggs (Pouillot et al. 2020).

An alternative option for New Zealand would be to consider introducing refrigeration and humidity controls throughout the egg supply chain. This approach would reduce the risk of *Salmonella* growth in egg contents, in the event that *S*. Enteritidis re-emerges on layer farms. If re-emergence occurs, eggs may be contaminated and consumers exposed before the issue is detected via on-farm testing. Refrigeration would also generally protect against growth of any *Salmonella* in egg contents that may have become contaminated via trans-shell transmission or egg cracks.

5.2.4 RMQ4: What is the best way to gather information on the prevalence of *Salmonella* in New Zealand eggs?

As discussed in the 2016 Risk Profile (Rivas et al. 2016) and elsewhere in this document, very low rates of surface contamination of eggs by *Salmonella* means that testing a large number of eggs would be necessary to achieve statistically valid results on the true prevalence of *Salmonella* in or on eggs. Contamination of eggs may be sporadic (for example, due to a contamination event such as a batch of contaminated feed leading to colonisation of some layer flocks), or chronic (for example, reoccurring in flocks due to *Salmonella* surviving cleaning regimes or being reintroduced by wildlife). Although not useful for informing the true prevalence of *Salmonella* in flocks and eggs, egg testing is useful for some purposes including assessing whether *S*. Enteritidis is transovarian, or for trace-back testing of eggs from a case to provide strong epidemiological evidence that the illness is associated with eggs.

The environmental testing of faeces and dust in the egg production environment has been shown to strongly correlate with the within-flock prevalence of *Salmonella* and the number of contaminated eggs produced (Wales et al. 2007, Carrique-Mas et al. 2008, Arnold et al. 2010,

Arnold et al. 2011, Dewaele et al. 2012b, Arnold et al. 2014). Thus, testing these sample types is more practical than sampling eggs, and forms the basis of most monitoring programs for *Salmonella* in the poultry industry, as described in Appendix C. EU sampling programs incorporate pooled faecal samples (cage flocks) or two pairs of boot swabs (barn and free range flocks), which also pick up dust, food, and other detritus. Some EU sampling programs replace a faecal sample or boot swab with a dust sample collected from different areas of the shed, or swabs of surfaces with visible dust present. The sensitivity of pooled faecal samples has been reported to increase with an increasing number of droppings in the sample, with 60 pinch samples of individual faeces predicted to reliably detect 5% flock prevalence of *Salmonella* (European Food Safety Authority Panel on Biological Hazards 2010a, Arnold et al. 2011). Dust samples have been found to be more sensitive than faecal samples for *Salmonella*, which is likely due to the organism being better able to survive in dry conditions (Haysom and Sharp 2003, Arnold et al. 2011). Therefore, the inclusion of this sample type increases the likelihood that early carriage and shedding of *Salmonella* by flocks will still be captured. Furthermore, surveys using a combination of both faecal and dust samples have been found to be the most sensitive at detecting *Salmonella* than either sample type individually (Arnold et al. 2010, Arnold et al. 2011, Schulz et al. 2011).

The experimental design for the 2016 cross-sectional survey of New Zealand layer flocks was based on the principles discussed above, and the testing schemes used in a number of other countries for environmental monitoring of laying flocks. In addition, the study sampled egg contact surfaces in egg collection and packing areas to indicate whether *Salmonella* is likely to be present on eggs. A similar survey could be conducted to investigate the current risk of *Salmonella* to New Zealand eggs, and assess how prevalence has changed compared with the 2016 baseline data. An additional option would be to conduct reactive sampling of eggs from flocks found to have a high prevalence of *Salmonella* in environmental samples. This would provide information on the risk that colonised flocks pose to eggs.

As discussed in Section 4.1 and Appendix C.1, environmental sampling of dust and faeces is also used under the current regulatory framework for *S*. Enteritidis (but not other *Salmonella* serotypes) in New Zealand poultry flocks. Rather than as an exercise to inform the risk to eggs per se, the main purpose of the testing is to detect and manage *S*. Enteritidis in flocks, to ensure that eggs produced by *S*. Enteritidis-positive flocks will not reach the consumer. As such, there is a higher concentration of testing at the apex of the supply chain, particularly, at the breeder flock level. In addition to selecting the most sensitive sample types and testing multiple samples, the programme involves sampling during times when the flock is most likely to be stressed and shedding, such as at the onset of lay. This recently implemented testing programme for *S*. Enteritidis in New Zealand breeder, layer and broiler flocks and hatcheries appears as rigorous as that conducted in the EU with respect to sampling frequency, timing, and sensitivity of sample types. However, the EU programme also regulates *S*. Typhimurium in all flocks and monitors three other serotypes in breeder flocks.

As discussed in Section 4.1.5, the current regulatory framework for *S.* Enteritidis might be also used to generate data on *Salmonella* prevalence and other serotypes of concern such as *S*. Typhimurium.

5.2.5 Risks associated with other foods

Since the 2016 Risk Profile, Risk Profiles (including updates) with *Salmonella* as the hazard have been produced for two important food transmission vehicles:

- Broiler chickens and poultry meat (Kingsbury 2023); and
- Raw milk (Soboleva 2019).

The 2023 Risk Profile concerning broiler chickens and poultry meat reported that the very low prevalence of *Salmonella* detected by the NMD programme suggests that the risk of salmonellosis from poultry meat remains low (Kingsbury 2023). The apparent increased frequency of poultry meat consumption has increased the risk of potential exposure, but this does not appear to have increased the risk of illness as reflected in salmonellosis notification rates. Earlier Risk Profiles considering *Salmonella* in poultry products were conducted before *S*. Enteritidis had been detected in poultry flocks in New Zealand. The 2023 Risk Profile concluded that the detection of the *S*. Enteritidis DT8, ST11 strain in poultry has the potential to increase the risk to the New Zealand broiler industry and to consumers of broiler poultry product. The potential for transovarian transmission of *S*. Enteritidis to eggs via the breeder flocks at the apex of the supply chain could result in widespread dissemination through the supply chain, and to consumers of contaminated poultry meat product. International studies indicate that *S*. Enteritidis might be more infectious, and higher hospitalisation rates suggest that this strain of *S*. Enteritidis poses a greater risk to human health compared with other *Salmonella* serotypes. There is also a risk to the international trade in hatching eggs and broiler product. The level of risk will be determined by the efficacy of the new control measures implemented to detect flock colonisation, manage colonised flocks, and control any dissemination of *S*. Enteritidis.

The raw milk Risk Profile considers pathogens in addition to *Salmonella*, and is an update to a 2013 Risk Profile (Soboleva 2013). The 2013 document reaffirmed earlier findings that the consumption of raw milk was a significant source of risk to human health, particularly in regard to food poisoning caused by Shiga toxin-producing *E. coli* and *Campylobacter*. The 2016 update reported that the main microbiological hazards present in raw milk in New Zealand have not changed since the 2013 risk assessment; salmonellosis was the fourth most notified enteric disease associated with the consumption of raw milk (Soboleva 2019).

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APPENDIX A: HAZARD AND FOOD

A.1 *SALMONELLA* GROWTH AND SURVIVAL

The following information is taken from a number of different sources but, unless otherwise referenced, is primarily derived from the *Non-typhoidal Salmonellae* datasheet prepared by ESR for MPI.[52] Content from the 2016 Risk Profile (Rivas et al. 2016) is also included.

A.1.1 Growth

Temperature: Some evidence for growth at temperatures <7˚C exists and 5.2 ˚C has been reported as the minimum growth temperature, but this is serotype specific, the data are still not universally accepted and doubts surrounding the experimentation exist. Growth greatly reduced at <15˚C. Maximum 49.5˚C. Optimum 35-37˚C.

pH: Minimum 3.8, optimum, 6.5-7.5, maximum 9.5. The minimum pH is influenced by other factors such as temperature, acid present, and the presence of salts and nitrate.

Atmosphere: Can grow in the presence or absence of air as a facultative anaerobe. *Salmonella* can grow in inoculated raw minced beef and cooked crab meat (stored at 8-11˚C) in the presence of 20-50% CO_2. The growth rate on beef muscle stored at 20˚C under nitrogen is only slightly less than that obtained when stored under air.

Water activity (a_w): Minimum 0.94, optimum 0.99, maximum >0.99.

A.1.2 Survival

Temperature: *Salmonella* survive well in the environment, on foods, human skin and other substrates. Survival is longer at chilled, compared with ambient, temperatures but is dependent on other factors such as pH and a_w. *Salmonella* can survive for long periods in frozen foods with a slow decrease in bacterial numbers due to cellular damage. Bacterial reduction is more rapid in the range 0 to 10ºC than in the range -17 to -20ºC. Some foods, including meat, ice cream and butter, appear to be protective of *Salmonella* during freezing and frozen storage. Rapid freezing promotes survival with lower frozen storage temperatures and less fluctuation giving greater survival (Jay et al. 2003). Frozen storage temperatures near 0˚C result in greater death or injury to bacterial cells.

pH: *Salmonella* are tolerant of acid conditions which is advantageous for survival in the environment and virulence.

Water activity: Survival in dry environments is a characteristic of these organisms. Some serotypes can survive for months or years in foods with a low a_w such as black pepper, chocolate, peanut butter and gelatine.

[52] https://www.mpi.govt.nz/dmsdocument/1214-Non-Typhoid-Salmonellae; accessed 9 December 2022

Biofilm production: Can form disinfectant and antibiotic-resistant biofilms which contribute to persistence in host, non-host and food-processing environments.

Viable but Non-Culturable (VBNC) state: Can transition to the VBNC state after exposure to low temperatures (5˚C) in nutrient-limiting microcosms for up to 300 days.

A.1.3 Inactivation

Temperature: Inactivation is greater during the freezing process compared with subsequent frozen storage, but those cells that survive remain viable. Freezing does not ensure the inactivation of *Salmonella* in foods.

Most serotypes are killed by normal cooking conditions (core temperature of 75˚C instantaneously or an equivalent time-temperature combination; for example, 70˚C for 2 minutes). In microbiological terms "D" refers to a 90% (a decimal or 1 $\log_{10}$ cycle) reduction in the number of viable organisms. D value temperature/time (˚C/minutes) in "all meats" include: $D_{60^\circ C}$ 12.2 minutes; $D_{65^\circ C}$ 2.1 minutes and $D_{70^\circ C}$ 0.4 minutes.

Some strains of some serotypes (for example, *S.* Senftenberg) are significantly more heat-resistant than the others when tested in culture, and this is influenced by the a_w, solutes and pH of the culture medium.

D-values for *Salmonella* can depend on the type of food involved. High fat and low moisture foods require more severe heat treatments to kill *Salmonella*; for example, in milk chocolate with <10% moisture, $D_{80^\circ C}$ for *S*. Typhimurium in milk chocolate is 222 minutes.

pH: *Salmonella* dies outside the ranges of pH permitting growth (<3.8 and >9.5). Inactivation depends on factors including the type of acid present and the temperature with the rate of death decreasing as the temperature is reduced; for example, inactivation is more rapid in commercial mayonnaise at 20ºC than it is at 4ºC.

In the studies by Alford and Palumbo, the authors demonstrated how decreasing temperature increases the inhibitory effects of pH and NaCl. In broth, at 10˚C, growth of 22/23 strains were inhibited by pH 5 and 2% NaCl (Alford and Palumbo 1969). At pH 5.8 (more representative of meat), 5% NaCl at 10˚C was required to inhibit growth. Increasing the salt concentration slightly decreased survival time at 10˚C.

Water activity: At a_w levels below those allowing growth (0.94), *Salmonella* dies slowly. The rate of death decreases as the a_w is lowered and also decreases as the temperature is reduced (Troller and Christian 1978).

Radiation: D-values in foods are between 0.5 kGy and 0.8 kGy, with values higher in dried foods. Radiation sensitivity is influenced by the substrate, temperature and the presence or absence of oxygen. UV and heat treatment applied together provide a synergistic, simultaneous lethal effect for *S.* Typhimurium and *S*. Enteritidis in broth culture.

Sanitisers and disinfectants: Most disinfectants commonly used in the food industry, are effective against *Salmonella* at recommended user concentrations. Some disinfectants have

a reduced effect, at recommended user concentrations against *Salmonella* on surfaces and in biofilms. Novel disinfectant strategies such as electrolysed water, antimicrobial materials and anti-biofilm–specific compounds have been shown to reduce or eliminate *Salmonella* under certain conditions.

Preservatives and other nonthermal processing technologies: *Salmonella* is sensitive to preservatives commonly used in foods. Growth is inhibited by benzoic, sorbic and propionic acid. Inhibition is enhanced by using a combination of factors; for example, the use of a preservative together with reduction in pH and temperature. High pressure processing (300 MPa, 35˚C, 1 minute) reduced *Salmonella* amounts on uncooked chicken breasts by 2 $\log_{10}$.

A.2 *SALMONELLA* TESTING AND TYPING METHODS

A.2.1 Serotyping

The 2011 Risk Profile (King et al. 2011b) described serotyping of *Salmonella* isolates; the same process is still conducted by the ESR ERL.

For some purposes, PCR-based methods are used for serotype. For example, laboratories that test poultry samples as part of the Emergency Control Scheme, may test these samples using an *S*. Enteritidis-specific PCR; a sample positive by this PCR screen must be reported as "Presumptive *Salmonella* Enteritidis" (Ministry for Primary Industries 2022f).

Another method for *Salmonella* serotyping involves computer (*in silico*) serotyping algorithms such as SeqSero2 (Zhang et al. 2019) or SISTR (Yoshida et al. 2016). These use WGS data to predict the serotype and have been shown to have a high level of accuracy relative to phenotypic testing. For example the SISTR algorithm correctly typed 94% of isolates (Uelze et al. 2020). As such, the approaches hold great promise in providing a direct replacement for prediction of individual somatic and flagella antigens, as currently defined by the Kaufmann White scheme.

A.2.2 Phage typing

Once the serotype is identified, a *Salmonella* isolate can be further subtyped by measuring susceptibility to a panel of bacteriophages, as described in the 2011 Risk Profile (King et al. 2011b). In New Zealand, the serotypes Typhimurium and Enteritidis, and the typhoidal serotypes Typhi and Paratyphi A and B, were usually phage typed. From 1 November 2019, the ESR ERL ceased all phage typing with all subsequent isolates being typed using WGS (see below).[53] Phage stocks are no longer available, and the method is being phased out internationally.

However, it should be noted that phage typing was reimplemented by the ESR ERL, at the request of MPI and the New Zealand Ministry of Health, for a selection of *S*. Enteritidis isolates from clinical, animal and poultry environment sources collected as part of the *S*. Enteritidis 2021 outbreak response (Ministry for Primary Industries 2021). However, the process is again discontinued with any additional isolates will be typed by whole genome sequencing.

[53] https://www.esr.cri.nz/our-research/nga-kete/infectious-disease-intelligence/enteric-reference-testing/human-salmonella-isolates/?we_objectID=5083; accessed 19 May 2023

A.2.3 Whole genome sequencing

From 1 November 2019, the ESR ERL carried out all *Salmonella* serotyping using WGS. Reporting now provides the serotype and Achtman 7-gene sequence type (ST). Such discrimination is important for investigating clusters of *Salmonella* isolates to determine if they are related, such as in outbreak investigations.

Finer subtyping of isolates of the same serotype and ST is achieved through SNP analysis. This approach provides high discriminatory power for microbial fine typing, as is necessary for outbreak investigations (Chattaway et al. 2019). As part of ongoing monitoring, each week the ESR ERL conducts a full cluster comparison of SNP differences looking for signals of an emerging outbreak.

Following the introduction of WGS, *Salmonella* subsp. (I) ser. 4,5,12:i:- is now reported as monophasic *S.* Typhimurium.

Note that there is no direct correlation between phage type and genomic SNP cluster type. A single phage type may comprise more than one SNP cluster type and are therefore not all related. Conversely, SNP clusters may comprise isolates of different phage types. This is not an error as phage type susceptibility for a given isolate is determined by its accessory genome which is not used in SNP analysis. This was seen with the poultry-associated strain *S.* Enteritidis SE_2019_C_01 where four apparent phage type case clusters (DT2, DT8, DT23 and DT28) were shown genomically to cluster as a single group (Jackie Wright, ESR, pers. comm).

A.3 ON FARM RISK FACTORS ASSOCIATED WITH OF *SALMONELLA* COLONISATION OF FLOCKS AND EGG CONTAMINATION

Colonisation of layer flocks and a contaminated layer environment are interconnected and both present a risk for contamination of eggs. Risk factors that influence flock colonisation and egg contamination on-farm are discussed below.

External shed environment: There are multiple potential sources of *Salmonella* in the environment surrounding the breeder, rearer and layer shed, such as wild birds, rodents, domestic and livestock animals. *Salmonella* can be transmitted into the shed via contaminated equipment or personnel. Risks can be minimised by adhering to strict biosecurity measures such as personnel/visitor control, minimising contact with domestic and wild animals, rodent control, upkeep of the immediate surroundings of the poultry house to minimise pest harbourage, and minimising movement of equipment and transport vehicles between farms and personnel. Various studies have highlighted rodents as a risk factor for *Salmonella* contamination of commercial layer flocks (Lapuz et al. 2012, Umali et al. 2012, Denagamage et al. 2016, Camba et al. 2020). Wild birds and foxes were reported to be important contributors of *S*. Typhimurium colonising a free range flock on an Australian layer farm (Chousalkar et al. 2016).

Internal shed environment: Significant sources of *Salmonella* within sheds include insects (for example, darkling beetles and flies) and rodents, as well as faeces from already colonised

birds. Because *Salmonella* can survive for extended periods in dust, *Salmonella* present in dust from previous flocks may act as a source for colonising subsequent flocks if shed cleanout and disinfection between flocks is inadequate. Various studies have reported that *Salmonella* can persist on farms through successive layer cycles, despite cleaning and disinfection procedures (Dewaele et al. 2012a, Dewaele et al. 2012b). This has also been reported in a New Zealand broiler house (Castañeda-Gulla et al. 2020). Multiple reservoirs and transmission routes were identified depending on the study, including accumulated organic matter and dust protecting the *Salmonella* from the disinfectants used, the egg collecting areas and rodents. The studies highlight the challenges of eliminating *Salmonella* from shed environments

Litter, which the birds are exposed to for the entire growing period, can harbour *Salmonella* from a previous flock and can result in contamination of the incoming flock; thus litter management is critical (Thippareddi et al. 2022). The New Zealand biosecurity manual recommends that dirty litter is removed between flocks and prior to shed sanitation (Poultry Industry Association of New Zealand 2015). Litter may be reused if there is a shortage of new litter or specific procedures to handle the reused litter are followed, but litter from a *Salmonella*-positive flock may not be reused.

Drinking water: Drinking water may pose a risk for *Salmonella* contamination if not of potable quality, or if *Salmonella*-containing biofilms have developed within the drinker water lines. Open troughs of drinking water can become contaminated by litter, feed, vectors and faeces. Furthermore, it may be difficult to prevent free-range chickens from accessing non-potable water sources such as transient puddles. The *Code of Welfare: Layer Hens (2018)* best practice recommendations include monitoring uncontrolled permanent water sources (for example, open stock troughs, creeks) used as major drinking water sources should be monitored for microbiological quality and palatability at a frequency dependent on test results (Ministry for Primary Industries 2018b). Furthermore, water within drinker lines should be regularly flushed.

Poultry feed: Poultry feed can act as a contamination source for both hens and eggs (Dewaele et al. 2012b, Li et al. 2012, Hsieh et al. 2014). Due to the ability of *Salmonella* to survive for long periods in a low water activity environment, it can survive for extended periods in dry poultry feed. Poultry feed and feed ingredients are widely traded between countries and feed has been implicated in the spread of certain *Salmonella* serotypes into new environments (European Food Safety Authority Panel on Biological Hazards 2019, Parker et al. 2022, Thippareddi and Singh 2022). Feed processing such as heat treatments during rendering of animal protein or pelleting should kill *Salmonella.* However, recontamination of feed from the feed processing environment can occur post-production, during transport to the broiler farm, or during storage on the farm.

In New Zealand, layer hens are fed using compound (multi-ingredient) feed that is either pelleted or served as mash. A pilot survey on selected finished animal feeds produced by feed mills across New Zealand from September 2014 to January 2015 included the testing of seven poultry feeds (mash and pelleted), all of which were negative for *Salmonella* (Rivas 2016). A 2016 survey of New Zealand layer farms also tested feed samples; 1/33 (3.0%) of samples

tested positive. While the majority of feed samples tested were collected from the farm level where possible (for example, feed silos), the positive sample was obtained from a laying shed from which other positive samples were also obtained (Kingsbury et al. 2019a). The isolate was of the serotype *S*. Thompson and other isolates from the shed were closely related, as ascertained via SNP analysis. Therefore, in this case, the feed may have become contaminated in the laying shed. Based on industry data, the most common *Salmonella* serotype in all finished animal feed in New Zealand prior to 2011 was *S*. Tennessee (Cressey et al. 2011). This serotype occurred infrequently amongst human cases, which argues against animal feed as a major source of human salmonellosis in New Zealand. However, the available information on *Salmonella* status of feed and feed ingredients in New Zealand is not sufficiently comprehensive to assess animal feed as a source of human salmonellosis cases.

There is a growing range of imported feed and feed ingredients entering New Zealand from a variety of overseas sources, which may pose an additional risk for the introduction of pathogens and contaminants into the food chain (Cressey et al. 2011). In New Zealand, it is recommended that feed is stored and managed in a manner that protects it from contamination (Ministry for Primary Industries 2022f). For feed that is purchased ready-made, the operator should obtain an assurance from their supplier that *Salmonella* is managed during feed processing (for example, by an appropriate heat treatment), and ideally, feed produced on-farm should be tested for *Salmonella*. Any contaminated feed should be re-processed or disposed of. If *S*. Enteritidis has been detected from feed, affected feed containers must be cleaned and sanitised (Ministry for Primary Industries 2022c).

Internationally, poultry feed has commonly been supplemented with sub-clinical levels of antibiotics to minimise pathogen colonisation, which is being phased out due to the increased awareness of the development of antibiotic resistance (Thippareddi et al. 2022). New Zealand takes a conservative approach to the use of antibiotics in poultry farming, and has adopted the following criteria for their use[54]:

- Evidence that such a use is consistent with accepted veterinary practice.
- Evidence that the use is linked to a specific etiologic microbiological agent or disease.
- Evidence that the use is appropriately targeted in poultry, and
- Evidence that no reasonable alternatives for intervention exist.
- That antibiotics of “critical importance” to human health are not used in poultry.

The major antibiotic used in broiler poultry in New Zealand was zinc bacitracin, but prophylactic use by broilers will cease this year (2023).[55] There has been more limited use of this antibiotic in layers and breeders compared with broilers (Kerry Mulqueen, PIANZ, pers. comm.). Zinc bacitracin was used as a prophylactic for the control of necrotic enteritis caused by a *Clostridia* species for which there is no poultry vaccine. This disease can affect the whole flock to kill 90% of the birds within 12 hours. The speed of transmission and death means that treatment after the flock is infected via adding antibiotics to feed or water is ineffective.

[54] https://www.pianz.org.nz/news/pianz-guidelines-for-the-use-of-antibiotics-in-poultry/; accessed 10 January 2023
[55] https://az659834.vo.msecnd.net/eventsairaueprod/production-nzvaevents-public/64c002bd04e348b5bb328686b9b3e2a8; accessed 26 January 2023

Other feed supplementation strategies that have been evaluated for reducing pathogen colonisation include the use of organic acids, probiotics, prebiotics, botanicals, bacteriocins and bacteriophages (Dunkley et al. 2009, Grant et al. 2016, Wessels et al. 2021, Thippareddi et al. 2022).

A.4 PREVALENCE OF *SALMONELLA* IN LAYING HENS OR EGG PRODUCTS IN NEW ZEALAND

A.4.1 NMD Programme

The NMD Poultry Programme monitors *Salmonella* (and *Campylobacter*) contamination of poultry carcasses at the end of primary processing (Ministry for Primary Industries 2022b). Poultry tested includes a bird of the following species that is intended for human consumption:

- a chicken of the species *G. gallus* (broiler chickens and EOL chickens);
- a duck of the species *Anas platyrhynchos domestica*, *Anas pekin* or *Cairina moschata*; or
- a turkey of the species *Meleagris gallopavo*.

For standard throughput premises operators (>1,000,000 birds processed per season), three carcass rinsates are collected for ducks, EOLs, meat chickens and turkeys each processing day. For very low throughput poultry premises (<1,000,000 birds processed per season) three carcasses of each type of bird are collected on one processing day each processing week. Very low throughput poultry premises processing multiple poultry types are required to sample three carcasses per processing week. One carcass rinsate of the three sampled is tested for *Salmonella*. If *Salmonella* is detected, isolates are sent to ESR ERL for further typing.

Of most relevance to this Risk Profile are data from EOL birds. Over the 2016-2022 period, these had a *Salmonella* prevalence of 2/1,476 (0.14%) from EOL samples (Table 10). Data from broilers, turkeys and ducks provide an indication of *Salmonella* prevalence in the wider poultry industry. Over the period 2015-2022 for meat chicken, 2016-2022 for turkey, and 2017-2022 for duck, there were 8/16,899 (0.05%) from meat chickens, 16/1,326 (1.21%) from turkey and duck samples. For meat chicken samples, there appeared to be a reduction in prevalence over time, with the highest detections in 2011 (0.25%) and 2012 (0.66%), down to no detections or one detection for the last four years; however, the number of detections were low for all years.

Table 10. *Salmonella* detections from NMD Programme testing of poultry samples (January 2015 to October 2022).

Year	Meat chicken samples			Duck and turkey samples[1]			End of Lay samples		
	Samples	Detections	% Detected	Samples	Detections	% Detected	Samples	Detections	% Detected
2015	2177	1	0.05%	ND	ND	ND	ND	ND	ND
2016	2208	2	0.09%	45	0	0.00%	49	0	0.00%
2017	2209	1	0.05%	253	2	0.79%	253	0	0.00%
2018	2202	2	0.09%	253	4	1.58%	301	0	0.00%
2019	2144	0	0.00%	255	3	1.18%	276	0	0.00%
2020	2116	1	0.05%	191	2	1.05%	258	0	0.00%
2021	2114	1[3]	0.05%	179	2	1.12%	220	1	0.45%
2022[4]	1729	0	0.00%	150	3	2.00%	119	1	0.84%
Total	***16899***	***8***	***0.05%***	***1326***	***16***	***1.21%***	***1476***	***2***	***0.14%***

[1] Due to the small number of turkey and duck farms, data were combined.

[2] ND No data.

[3] This isolate was *S.* Enteritidis.

[4] Data were for January to October 2022.

A.4.2 Common serotypes present in New Zealand poultry

Annual data for the prevalence of *Salmonella* in poultry from the poultry industry, as reported in the *Surveillance* biosecurity magazine published by MPI, is presented in Table 11.[56]

[56] https://www.mpi.govt.nz/biosecurity/about-biosecurity-in-new-zealand/surveillance-biosecurity-magazine/; accessed 2 March 2023

Table 11. *Salmonella* serotypes identified 10 or more times from poultry industry data. Isolates were from poultry feed, broiler samples, or the environment (env) (2015 to 2021).[1]

Serotype	2015		2016		2017		2018		2019		2020		2021		Total
	Feed	Broiler/ Env	Feed	Broiler/ Env	Feed	Broiler/ Env	Feed	Broiler/ Env	Feed	Broiler/ Env	Feed	Broiler/ Env	Feed	Broiler/ Env	
Mbandaka		1	1		81		5	46				1	16	5	***156***
Bovismorbificans		85		22		29		4		1				3	***144***
Enteritidis														123	***123***
Infantis	6	10		9		12	2	9		15		8		19	***90***
Agona		10	1	7		6		24	6	17		5	3	6	***85***
Typhimurium	17	8	6	2	4	1		3		2	1	8	9	23	***84***
Livingstone		4				11		16		15		29		1	***76***
Senftenberg	1	1		8	2	7		13	1	21		6	6	8	***74***
Derby				4	11	3		3	2	2		1	1	2	***29***
Havana									28						***28***
Rissen				1	1	2		1		1			9	11	***26***
Stanley			2					3		8		8		2	***23***
Total positive	***30***	***135***	***16***	***59***	***103***	***90***	***13***	***140***	***53***	***98***	***5***	***83***	***95***	***273***	
Total samples tested	***3,578***	***3,831***	***4,150***	***3,683***	***3,232***	***3,894***	***2,877***	***3,142***	***3,699***	***1,781***	***2,632***	***1,459***	***7,250***	***3,1628***	

[1] Data was sourced from *Surveillance* magazine annual reports. https://www.mpi.govt.nz/biosecurity/about-biosecurity-in-new-zealand/surveillance-biosecurity-magazine/

A.5 OVERSEAS DATA: *SALMONELLA* IN AND ON EGGS

A.5.1 Prevalence of *Salmonella* in layer flocks in other countries

Data showing *Salmonella* prevalence from layer flocks from other countries, are summarised in Table 12 which focusses on studies published between 2016 and April 2023. These data also inform on sample types and serotypes associated with layer flocks in different regions. *Salmonella* prevalence differed considerably between samples and studies based influenced by the methodology applied and risk factors associated with different regions. In addition, some studies were longitudinal surveys of individual layer flocks while others conduct surveys of multiple flocks. Serotypes also differed considerably in different regions.

A.5.2 EU verification testing of breeder and layer flocks

The verification testing for layer flocks and eggs is described in Appendix C. Surveillance data are compiled annually by EFSA (European Food Safety Authority and European Centre for Disease Prevention and Control 2022). Data for 2016 to 2021 EU verification testing of layer flocks has been collated in Table 12.

For countries running the regulation (EC) No 2160/2003, 2021 control programme, the 2021 prevalence of *Salmonella* in *G. gallus* breeding flocks (which includes breeders for both broiler and layer flocks) was 348/13,983 (2.5%). This was similar to prevalence data for 2020 (2.0%) and 2019 (2.3%). Of these, 81 (0.58%) were positive for any of the five target serotypes, compared with 0.52% in 2020 and 0.62% in 2019. The most frequently reported target serotype was *S*. Enteritidis with 55 positive flocks (0.39%). There were 29 breeder flocks positive for *S*. Enteritidis in 2020 and 53 in 2019. For the other target serotypes, there were 15 (0.11%) flocks positive for *S*. Typhimurium, two (0.01%) for *S*. Hadar, six (0.04%) for *S*. Infantis and three (0.02%) for *S*. Virchow. There has been an overall decreasing trend for the *Salmonella* prevalence as well as target serotype prevalence in EU breeder flocks since testing was implemented in 2007.

The 2021 EU prevalence of *Salmonella* in layer flocks was 1,323/39,546 (3.3%), which was similar to prevalence in 2020 (4.0%) and 2019 (3.9%). The prevalence of layer flocks that were positive for either of the two target *Salmonella* serotypes was 533/39,546 (1.3%), which was the same as previous years (1.3% in 2020 and 2019). Of the two target serotypes, the prevalence was higher for *S*. Enteritidis which accounted for 76.4% of flocks positive for target serotypes (1.0% of total flocks), whereas *S*. Typhimurium accounted for 23.6% (0.3% of total flocks). In 2021, seven member states did not meet the required 2% reduction targets for layer hens. Since the testing programme began in 2008, modelling has shown a significant reduction in prevalence of target serotypes. The estimated EU prevalence of *Salmonella*. in laying hen flocks was 7.0% (95% CI 4.3; 11.3) in 2008 and decreased to 2.1% (95% CI 1.4; 3.2) in 2014, with a steep downturn. During the following years, it increased and reached 2.6% (95% CI 1.7; 4.0) in 2021.

Table 12. Prevalence of *Salmonella* in layer flocks from New Zealand and other countries (published since 2016).

Year	Country/ region	Sample type	Prevalence	*Salmonella* serotype/s[1]	Reference
2016	New Zealand (32 farms, 67 flocks)	dust	19/67 (28%)	Infantis (44%), Thompson (35%), Typhimurium (14%), Anatum (5%), Mbandaka (2%)	(Kingsbury et al. 2019a)
		faeces	7/67 (10%)		
		manure belt/boot swabs	11/67 (16%)		
		feed	1/33 (3%)		
		packhouse egg contact surfaces	5/87 (6%)		
2014-2018	Australia (longitudinal study)	boot swab	184/400 (46%)	Typhimurium (10%), Infantis (15%), Singapore, Agona, Virchow	(Crabb et al. 2019a)
		dust swab	376/1,002 (38%)		
		manure belt swab	276/715 (39%)		
		egg belt swab	228/762 (30%)		
		Total	1,074/2,879 (37%)		
2014-2015	Australia (free range flocks)	dust	14.2% (CI 10.6-18.9)	Mbandaka, Typhimurium, Agona, Anatum, Worthington, Singapore, Infantis	(Gole et al. 2017)
		boot swabs (range)	12.5% (CI 5.9-23.9)		
		boot swabs (shed)	3.6% (CI 0.3-12.8)		
		faeces	11.8% (CI 8.5-16.1)		
		ramps	8.2% (CI 5.5-12.1)		
		nest boxes	7.9% (CI 5.2-11.7)		
		egg belts	5.4% (CI 3.2-8.7)		
Not provided	Australia (caged flock A; longitudinal)		proportion positive:	Typhimurium and non-Typhimurium	(McWhorter and Chousalkar 2020)
		dust swab	0.97±0.02		
		faeces	0.69±0.03		
		egg belt swab	0.98±0.01		
		egg suction cup swab	0.13±0.07		
	(caged flock B; longitudinal)	dust swab	0.91±0.03		
		faeces	~0.45[2]		
		egg belt swab	~0.70[2]		
2011-2012	Canada (21 farms, 48 flocks)	manure belt swab	26/48 (54%)	Heidelberg (18%), Kentucky (18%), Mbandaka (16%), Agona (12%), Alachua (8%), Braenderup (6%), Hadar (4%), Schwarzengrund (4%), 6 others at 2% each	(St Amand et al. 2017)
		feeder swab	23/48 (48%)		
		feed motor swab	22/48 (46%)		
		egg belt and wall swab	20/48 (42%)		
		fan swab	14/40 (35%)		
		cage bottom swab	15/48 (31%)		
		lobby swab	13/48 (27%)		
2013-2017	Canada (multiple farms)	faeces (9 farms, Ontario)	11/38 (29%)	Braenderup, Heidelberg, Kentucky, Ohio, Livingstone, Infantis, Liverpool, Mbandaka	(Agunos et al. 2021)
		faeces (15 farms, Ontario)	5/60 (8%)		
		environment sponge swab (26 farms, British Columbia)	4/54 (7%)		
2011-2012	Ghana	boot swab, dust, feed, drinking water (samples were from both layer and broiler flocks)	94/200 (47%)	16 serotypes; most prevalent were Kentucky (18%), Nima (13%), Enteritidis (11%), Muenster (11%)	(Andoh et al. 2016)
	Korea	faeces	28/67 (42%)		(Im et al. 2015)

Year	Country/ region	Sample type	Prevalence	*Salmonella* serotype/s[1]	Reference
2013-2014	(32 farms, 67 flocks)	dust	27/67 (40%)	15 serotypes, most prevalent were Bareilly, Mbandaka, Rissen, V1,13,22:i:-, Agona, Infantis, Saintpaul	
2012-2013	Nigeria (523 farms)	dust	63/523 (12%)	82 serotypes, most prevalent were Kentucky, Poona, Elisabethville, Larochelle, Agama	(Fagbamila et al. 2017)
		boot swabs of litter	67/523 (13%)		
		faeces	75/523 (14%)		
		feed	73/523 (14%)		
		water	52/523 (10%)		
2021	Europe	Regulatory testing (Regulation (EC) No 2160/2003, 2021)[3]	1,323/39,546 (3%); 1% positive for target serotypes	Target serotypes: Enteritidis (31%); Typhimurium (10%)	(European Food Safety Authority and European Centre for Disease Prevention and Control 2022)
2020	Europe	Regulatory testing (Regulation (EC) No 2160/2003)	1,389/~34,725[4] (4%); 1% were positive for target serotypes	Top serotypes: Enteritidis, Typhimurium (including monophasic), Infantis, Derby	(European Food Safety Authority and European Centre for Disease Prevention and Control 2021b)
2019	Europe	Regulatory testing (Regulation (EC) No 2160/2003)	1,529/~39,205 (4%); 1% were positive for target serotypes	Target serotypes: Enteritidis (24%), Typhimurium (including monophasic) detected(8%)	(European Food Safety Authority and European Centre for Disease Prevention and Control 2021a)
2018	Europe	Regulatory testing (Regulation (EC) No 2160/2003)	1,539/~38,475[3] (4%); 1% were positive for target serotypes	Target serotypes: Enteritidis (20%); Typhimurium (including monophasic) (7%)	(European Food Safety Authority and European Centre for Disease Prevention and Control 2019)
2017	Europe	Regulatory testing (Regulation (EC) No 2160/2003)	1,361/~36,784[3] (4%); 1% were positive for target serotypes	Target serotypes: Enteritidis (24%), Typhimurium (6%)	(European Food Safety Authority and European Centre for Disease Prevention Control 2018)
2016	Europe	Regulatory testing (Regulation (EC) No 2160/2003)	4%; 1% were target serotypes	Target serotypes: Enteritidis (1.2% flock prevalence), Typhimurium (0.2% flock prevalence)	(European Food Safety Authority and European Centre for Disease Prevention and Control 2017)

[1] Serotype percentages are relative to all *Salmonella* isolates unless specified otherwise.
[2] Proportions were estimated from a graph.
[3] Regulatory testing is described in Appendix C.
[4] The number sampled was not provided, but was estimated from the percentage positive.
[5] The reporting by EFSA of serotype data from layer flocks differed year-to-year; for some years, percentages of the target serotypes were provided; and other years, the EU top 5 serotypes reported.

A.5.3 Prevalence of *Salmonella* in and on eggs in other countries

Data on the prevalence and serotypes of *Salmonella* isolated from eggshells and egg contents that has been published since 2016 is presented in Table 13 (Appendix).

Australian studies assessed in the earlier Risk Profiles did not detect *Salmonella* in the contents of eggs at either the farm or at retail. However, in a more recent study, *S.*

Typhimurium and *S*. Infantis were both detected from internal contents of eggs sampled directly from Australian egg farms, which was the first report of *Salmonella* detection in egg contents from commercial production in Australia (Crabb et al. 2019b). *Salmonella* was detected at no more than 1 CFU/ml. The true prevalence of *Salmonella* in eggs was the highest at the onset of lay. A higher egg prevalence was also associated with a lower body weight, higher egg production, higher egg weight and mass than the breed standard for age, and poorer feed conversion efficiency. The ability to detect *Salmonella* from egg contents in this study but not previous studies, may have been due to the age and *Salmonella*-shedding status of the laying hens from which the eggs were derived, testing of a larger number of eggs by that study (8958 egg samples were tested), or differences in testing methodology between studies. Note that presence of *Salmonella* in contents of freshly laid eggs does not inform on whether these isolates or serotypes are able to survive in egg albumen during storage. An inability to survive in albumen could account for detection in freshly laid eggs in this study but absence in eggs from retail from previous Australian studies.

Another recent study detected *Salmonella* contamination from both eggshells and contents from retail in Western Australia (Sodagari et al. 2019). *Salmonella* was isolated from 4.5% (9/200) of eggshells and 3% (6/200) of egg contents. Isolates were either *S*. Typhimurium or *S*. Infantis. There were more isolations of *S*. Typhimurium (5 isolations) than *S*. Infantis (1 isolation) from egg contents. Based on the serotypes present (that is, non-Enteritidis), egg content contamination likely occurred via trans-shell rather than transovarian transmission, but the authors did not specifically address this.

Prevalence of *Salmonella* in or on eggs, sourced from either layer farms or at retail from various other countries, is also provided in Table 13. The prevalence values vary widely, which in part would reflect different methodologies; for example, the number of eggs included in each sample, and whether they were collected from retail or the laying shed. Prevalence on egg surfaces/eggshells ranged from 0-17%. Prevalence from egg contents ranged from 0-12%.

Table 13 also includes data from EU regulatory testing of eggs from 2016 to 2021. Where *Salmonella* prevalence from table eggs was reported, prevalence ranged from 0.1-0.4%. When data was reported for eggs and egg products (non-RTE), prevalence ranged from 0.6% to 0.8%. The most prevalent serotype from these product types was *S*. Enteritidis.

Table 13. Prevalence of *Salmonella* in and on eggs from countries other than New Zealand (published since 2016).

Year	Country/ region	Sample location	Sample type[1]	Prevalence (%)	*Salmonella* serotype/s	Reference
2016	Australia	Retail	Contents	0/668 (4,008 total eggs) (0%)		(Symes et al. 2016)
2017-2018	Australia	Retail	Eggshells, crushed	9/200 (2,400 total eggs) (5%)	Typhimurium, Infantis	(Sodagari et al. 2019)
			Egg contents	6/200 (2,400 total eggs) (3%)		
2001-2011	Australia	Farms associated with outbreaks (caged, free-range and barn)	Whole eggs	Detected from eggs from 16/49 farms; egg sampling methodology and prevalence not reported	Typhimurium, Infantis, Singapore, Orion, Montevideo, Anatum, Mbandaka, Tennessee	(Moffatt et al. 2017)
2014-2015	Australia	Farm (free-range)	Egg external surface (floor eggs)	3.6% (CI, 1.9-6.5%)	Not reported, but Mbandaka, Typhimurium, Agona, Anatum, Worthington, Singapore present in flocks	(Gole et al. 2017)
			Egg external surface (nest eggs)	0.6% (CI, 0.2-1.4%)		
			Egg contents	0%		
Not reported	Australia	Farm (caged and free-range)	Egg external surface	TP[2]: 0.014 (95% CI 0.005, 0.038)	Typhimurium (52%), Infantis (39%)[3]	(Crabb et al. 2019b)
			Shell and membrane	TP: 0.01 (95% CI 0.003, 0.032)		
			Yolk and albumen	0.007 (95% CI 0.001, 0.027)		
Not reported	Australia	Farm (caged)	Eggs: Flock A	Proportion: 0.14±0.04; Mean numbers: 77.6±74.5 MPN/ml	Not reported, but Typhimurium and non-Typhimurium present in flock[3]	(McWhorter and Chousalkar 2020)
			Eggs: Flock B	Proportion: 0.06±0.03; Mean numbers: 4.0±1.7 MPN/ml		
Not reported	Australia	Farm (free-range)	Eggs	Proportion: <0.05	Not reported, but Typhimurium detected in flock	(McWhorter and Chousalkar 2019)
Not reported	US	Farm with experimentally infected indoor, cage-free flocks	Egg contents	*S.* Enteritidis-infected flock: 35/1026 (3%) *S.* Typhimurium-infected flock: 15/1264 (1%)	Enteritidis, Typhimurium (flocks were infected with these serotypes)	(Gast et al. 2021)
2014-2015	US	Farms and farmers markets	Egg surfaces	9/252 (504 total eggs) (3.6%)	Not reported	(Kilonzo-Nthenge et al. 2016)
			Egg contents	0/252 (504 total eggs) (0%)		
2021	Europe: 18 MS	Regulatory testing[5]	Eggs and egg products (non-RTE)	53/6,501 (0.8%)	Not reported	(European Food Safety Authority and European Centre for Disease

Year	Country/ region	Sample location	Sample type[1]	Prevalence (%)	*Salmonella* serotype/s	Reference
						Prevention and Control 2022)
2020	Europe: 15 MS	Regulatory testing	Eggs and egg products (non-RTE)	35/5,554 (0.6%)	Not reported	(European Food Safety Authority and European Centre for Disease Prevention and Control 2021b)
2019	Europe: 11 MS	Regulatory testing	Eggs and egg products (non-RTE)	6/5,051 (0.8%)	Enteritidis (50%)[4]	(European Food Safety Authority and European Centre for Disease Prevention and Control 2021a)
	8 MS		Table eggs	6/4,493 (0.1%)		
2018	Europe: 13 MS	Regulatory testing	Table eggs	23/6,252 (0.4%)	Enteritidis (58%), Infantis also reported[4]	(European Food Safety Authority and European Centre for Disease Prevention and Control 2019)
2017	Europe: 15 MS	Regulatory testing	Table eggs	29/9,700 (0.3%)	Not reported	(European Food Safety Authority and European Centre for Disease Prevention Control 2018)
2016	Europe	Regulatory testing	Table eggs	~17[6]/5,782 (0.3%)	Not reported	(European Food Safety Authority and European Centre for Disease Prevention and Control 2017)
Not reported	Brazil	Retail	Eggshells and yolks	2/160 (480 total eggs) (1.25%)	Panama (from eggshell), Gallinarum (from egg yolk)	(Haubert et al. 2022)
2016	China	Retail (multiple types)	Egg external surface	19/5,548 (33,288 total eggs) (0.3%)	Most prevalent: Enteritidis (46%), Typhimurium (11%), Also reported: Bonn, Choleraesuis, Infantis, Iomita, Narashino, Rissen, Stanley, Tarshyne, Typhi, Virchow	(Li et al. 2020b)
			Egg contents	9/5,548 (33,288 total eggs) (0.2%)		
2013-2014	China	Retail, farm	Egg contents	46/814 (5.6%)	Most prevalent: Typhimurium (25%), Indiana (23%), Thompson (13%), Enteritidis (11%); nine additional serotypes (2–5%)	(Li et al. 2020a)

Year	Country/ region	Sample location	Sample type[1]	Prevalence (%)	*Salmonella* serotype/s	Reference
2021	China	Retail	Egg contents	16/130 (12.3%)	Typhimurium (100%)	(Li et al. 2022)
2012-2013	Ethiopia	Farms, retail (market)	Egg external surface	8/300 (2.7%)	Not reported	(Kemal et al. 2016)
			Egg contents	0/300 (0%)		
Not reported	Korea	Retail, farm	Egg external surface	0/475 (0%)	Not reported	(Lee et al. 2016)
2013-2014	Korea	Farms	Eggshells	10/58 (17.2%)	Bareilly, Mbandaka, Heidelberg, Infantis, Braenderup	(Im et al. 2015)
			Egg contents	3/58 (5.2%)		
2010-2012	South Korea	Grading and packing facility	Egg contents	9/200 (4,000 total eggs) (4.5%)[7]	Not reported	(Lee et al. 2017)
2021	Morocco	Retail (formal and informal)	Egg external surface	~12/590 (1770 total eggs) (2%)	*Salmonella* isolates referred to as *S. enteritidis*, but no serotyping was reported and this may instead refer to *S. enterica*	(El Ftouhy et al. 2022)
			Contents	0/590 (1770 total eggs) (0%)		
Not reported	Nigeria	Retail, farm	Eggshells	13/100 (500 total eggs) (13%)	Agama, Colorado, Lattenkamp, Kingston, Kentucky, Durham, Bradford, Derby, Kentucky, Carno	(Agbaje et al. 2021)
			Egg contents	1/100 (500 total eggs) (1%)	Alachua	
2016-2018	Romania	From producer	Eggshells	3/48 (6.3%)	Enteritidis (67%), Infantis (33%)	(Tîrziu et al. 2020)
2018	Zambia	Retail	Egg external surface	5/216 (1080 total eggs) (2.3%)	Not reported	(Kapena et al. 2020)
			Egg contents	0/216 (0%)		

[1] Egg surface and eggshell sampling differed; eggshell sampling referred to the whole shell (external and internal). Egg samples were typically tested in pools of multiple eggs; for example, multiples of five, six or twelve eggs, depending on the experiment. When pooled eggs were tested, the total number of eggs sampled are indicated.

[2] Abbreviations: TP: True prevalence, which is a measure of apparent prevalence with a correction applied based on predicted imperfect diagnostic test performance. RTE, ready-to-eat. MS, member states. CI, confidence interval.

[3] Other serotypes were not tested for.

[4] Only the EU top five serotypes were reported.

[5] The number of eggs tested per sampling unit was not reported.

[6] The number positive was an estimate calculated from the percentage positive and number tested.

[7] Prevalence differed by testing method; results were highest by direct plating (data shown).

APPENDIX B: EVALUATION OF ADVERSE HEALTH EFFECTS

B.1 ANTIMICROBIAL RESISTANCE OF NEW ZEALAND *SALMONELLA* ISOLATES

For the time period considered in this report for which antimicrobial susceptibility data was available (2015 to 2019), ESR tested the antimicrobial resistance of approximately 20% of all human and non-human non-typhoidal *Salmonella* isolates received for typing.[57] In addition, all isolates of phage types that were internationally recognised as being multidrug-resistant were tested. These included the *S. enterica* serotype 4,[5],12:i:- and *S.* Typhimurium phage types DT12, DT104, DT120, DT193 and U302. Testing was conducted yearly for the multiresistant phage types. For the other non-typhoidal *Salmonella*, testing was conducted for the years 2015, 2016 and 2019.[58,59]

Resistance to the 13 antimicrobials tested and multiresistance to three or more of these is shown for the years 2015 to 2019 for human isolates in Table 14 and for non-human isolates (which included isolates from animals, food and environmental samples) in Table 15. Note that the panel and number of antimicrobials differed by testing year. For each year of testing, *Salmonella* from human sources were significantly ($p < 0.05$) more resistant to at least three of the antibiotics tested than *Salmonella* from non-human sources; ampicillin was identified in each year. For example, in 2019, *Salmonella* from human sources were significantly more resistant to ampicillin, amoxicillin-clavulanate, ciprofloxacin, streptomycin and sulphonamides than *Salmonella* from non-human sources, and this was independent of a history of overseas travel.

The percentage of non-typhoidal *Salmonella* isolates (human and non-human data combined) that were resistant to three or more antimicrobials was low each year (usually less than 6%). Between 2015 and 2019, the percentage of isolates from humans that were resistant to three or more antimicrobials was between 5.1 and 7.7 per year. For non-human isolates this range was 0.0-1.5%. When the human and non-human isolates were combined, the percentages that were fully susceptible to all tested antimicrobials each year were high: 89.3% (2015), 90.0% (2016) and 91.0% (2019).

[57] Data are available from the annual reports of antimicrobial susceptibility among *Salmonella*, produced by ESR and available at: https://surv.esr.cri.nz/antimicrobial/salmonella.php (accessed 2 November 2022).
[58] The change in typing from phage typing to whole genome sequencing impacts the priority list of isolates which are tested phenotypically for antimicrobial resistance.
[59] Funding for testing of non-human *Salmonella* isolates ceased at the end of 2019; only human isolates are currently being tested for antimicrobial susceptibility.

Table 14. Antimicrobial resistance of a sample of New Zealand non-typhoidal *Salmonella* isolates from humans, 2015-2019.[1]

Antimicrobial	Percent of isolates resistant each year (n=number tested)		
	2015 (n=235)	2016 (n=237)	2019 (n=225)
Ampicillin	10.2	5.9	6.2
Amoxicillin-clavulanate	2.6	1.3	2.7
Cefotaxime	ND[4]	ND	0.4
Ceftazidime	ND	ND	0.4
Cephalothin	2.1	0.0	ND
Chloramphenicol	2.6	2.5	1.8
Ciprofloxacin[2]	0.0	5.9	6.7
Co-trimoxazole	3.0	3.4	2.7
Gentamicin	0.9	0.4	0.9
Streptomycin	6.0	4.2	4.4
Sulphonamides	6.8	6.3	5.8
Tetracycline	7.7	6.8	6.2
Trimethoprim	3.0	ND	ND
Multiresistant to ≥3 antimicrobials[3]	7.7	5.1	6.2

[1] Data source: https://surv.esr.cri.nz/antimicrobial/salmonella.php; accessed 6 March 2023.

[2] The ciprofloxacin resistance rates for 2015 are based on ciprofloxacin disc susceptibility testing and the current CLSI breakpoints. The rates for 2016 and 2019 are based on testing with the surrogate pefloxacin disc and EUCAST breakpoints.

[3] For estimates of multidrug resistance, and co-trimoxazole and trimethoprim resistance, were counted as a single resistance (for years that both antibiotic susceptibilities within a pair were tested).

[4] ND Not determined.

Table 15. Antimicrobial resistance of a sample of New Zealand non-typhoidal *Salmonella* isolates from food, animal and environmental samples, 2015-2019.[1]

Antimicrobial	Percent of isolates resistant each year (n=number tested)		
	2015 (n=120)	2016 (n=133	2019 (n=175)
Ampicillin	0.0	0.8	0.0
Amoxicillin-clavulanate	0.0	0.8	0.0
Cefotaxime	ND[4]	ND	0.0
Ceftazidime	ND	ND	0.0
Cephalothin	0.0	0.0	ND
Chloramphenicol	0.0	0.0	0.0
Ciprofloxacin[2]	0.0	0.8	0.0
Co-amoxiclav	ND	0.0	ND
Co-trimoxazole	0.0	0.8	0.6
Gentamicin	0.0	0.0	0.0
Streptomycin	0.8	0.8	0.6
Sulphonamides	2.5	1.5	0.6
Tetracycline	1.7	2.3	6.9
Trimethoprim	0.0	ND	ND
Multiresistant to ≥3 antimicrobials[3]	0.0	1.5	0.0

[1] Data source: https://surv.esr.cri.nz/antimicrobial/salmonella.php; accessed 6 March 2023.
[2] The ciprofloxacin resistance rates for 2015 are based on ciprofloxacin disc susceptibility testing and the current CLSI breakpoints. The rates for 2016 and 2019 are based on testing with the surrogate pefloxacin disc and EUCAST breakpoints.
[3] For estimates of multidrug resistance, co-trimoxazole and trimethoprim resistance, were counted as a single resistance (for years that both antibiotic susceptibilities within a pair were tested).
[4] ND Not determined.

Trends in antimicrobial resistant *Salmonella* from human cases for the years 2011 to 2019 are shown in Figure 9. As noted in the 2019 report,[60] there has been a significant decrease ($p < 0.05$) in resistance for ampicillin, streptomycin, sulphonamides, and tetracycline since 2011. There has been a significant increase ($p < 0.05$) in resistance for amoxicillin-clavulanate and ciprofloxacin which may be related to a method change issued by the Clinical and Laboratory Standards Institute (CLSI) to European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard, which was introduced in 2016. The increase in ciprofloxacin resistance may be partially attributable to the change in test methods from 2016, as the use of the surrogate pefloxacin disc detects more low-level ciprofloxacin resistance than testing with ciprofloxacin itself.

For the time period 2015 to 2019, *Salmonella* isolates from salmonellosis cases reported to have travelled overseas were significantly more resistant to at least one antimicrobial than isolates from cases for whom no recent overseas travel was reported.

[60] https://surv.esr.cri.nz/PDF_surveillance/Antimicrobial/SAL/SAL_2017-2019.pdf, accessed 7 November 2022.

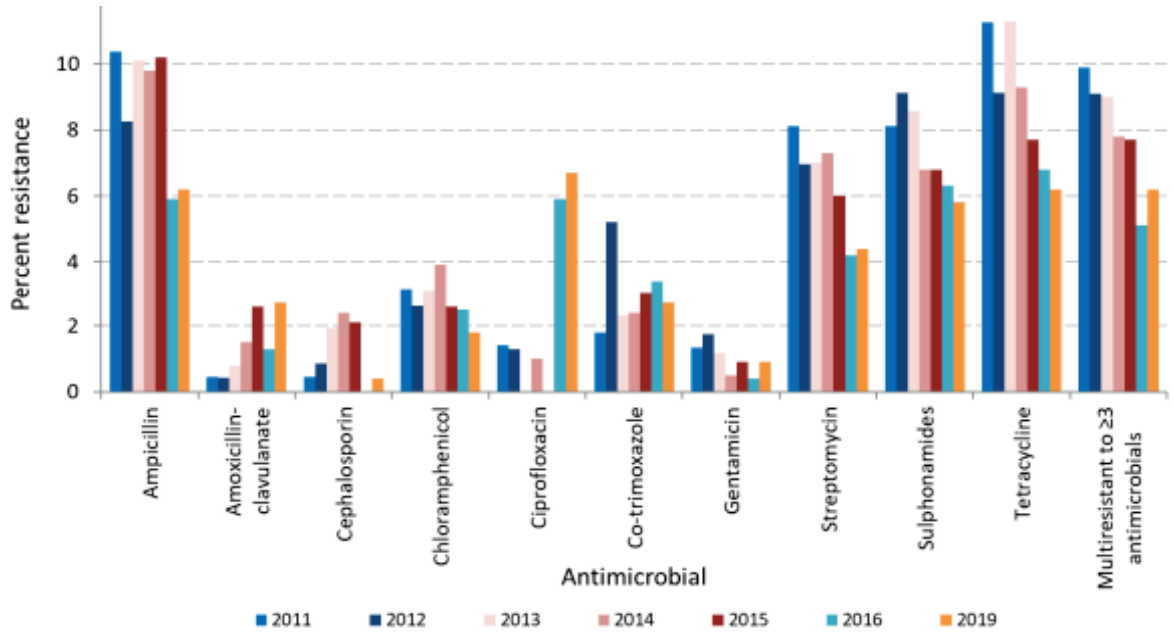

Figure 9. Resistance among non-typhoidal *Salmonella* from human cases, 2011 to 2019. Graph reproduced from ESR (2019).[61]

- The ciprofloxacin resistance rates for the years 2011 to 2015 are based on ciprofloxacin disc susceptibility testing and the current CLSI breakpoints. The rates for 2016 and 2019 are based on testing with the surrogate pefloxacin disc and EUCAST breakpoints. Testing with a pefloxacin disc is more likely to detect low-level ciprofloxacin resistance than ciprofloxacin disc susceptibility testing. This change in test procedures is likely to account for the apparent increase in ciprofloxacin resistance from 2016.
- The cephalosporin resistance rates for the years 2011 to 2016 are based on cephalothin (1st generation cephalosporin) disc susceptibility testing. The rates for 2019 are based on cefotaxime and ceftazidime (3rd generation cephalosporin) disc susceptibility testing. This change in test procedure may be responsible for the apparent decrease in cephalosporin resistance.

The prevalence and multiresistance status of all isolates belonging to internationally recognised multiresistant *S.* Typhimurium phage types during the period 2015 to 2019 is shown in Table 16. These phage types include *S.* Typhimurium phage types DT104, U302, DT12, DT120 and DT193, which are characterised by resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracycline. In addition, *S. enterica* serotype 4,[5],12:i:- (which is considered a monophasic variant of *S*. Typhimurium) is tested; isolates of which are typically multiresistant to ampicillin, streptomycin, sulphonamides and tetracycline. *S. enterica* serotype 4,[5],12:i:- was the most commonly reported type from this group each year, and isolate numbers appear to be increasing (21 in 2010; 57 in 2019 when it was the third most common serotype). The majority of isolates of this serotype were multiresistant.

[61] https://surv.esr.cri.nz/PDF_surveillance/Antimicrobial/SAL/SAL_2017-2019.pdf, accessed 7 November 2022.

Table 16. Prevalence of known multiresistant *S.* Typhimurium phage types and the 4,[5],12:i:- serotype in New Zealand (isolates from humans, environmental sources, food and animals) for the years 2015 to 2019.[1]

Type	Number of isolates of type that tested multiresistant/number of isolates of type (number of multiresistant isolates where overseas travel was identified)[2]				
	2015	2016	2017	2018	2019
DT104	1/1	0/0	0/0	0/0	0/0
U302	0/0	0/1	0/1	1/1 (1)	0/0
DT120	0/0	1/3 (1)	3/4 (2)	1/3 (1)	1/3
DT193	7/18	0/9	3/25 (1)	3/7 (1)	5/12
DT12	0/0	0/11	0/9	0/0	2/8 (2)
4,[5],12:i:-	27/33 (23)	30/34 (20)	29/36 (19)	23/26 (18)	41/57 (21)
Total	***35/52 (23)***	***31/58 (21)***	***35/75 (22)***	***28/37 (21)***	***49/80 (23)***

[1] Data source: https://surv.esr.cri.nz/antimicrobial/salmonella.php; accessed 6 March 2023.

[2] Travel status of cases is not always reported.

B.1.1 Antimicrobial resistance among *Salmonella* isolates from eggs in other countries

Various international studies that have examined *Salmonella* prevalence in and on eggs (Table 13) have also investigated the antimicrobial resistance of isolates. Some examples include:

- An Australian survey isolated *S*. Typhimurium and *S*. Infantis from the surfaces and contents of retail eggs (Sodagari et al. 2019). Two *S*. Typhimurium isolates were resistant to ampicillin, of which one carried β-lactamase resistance gene *blaTEM-1b*.
- Another Australian study examined antimicrobial resistance from 307 isolates comprising 30 serotypes that were isolated from commercial layer flocks (Veltman et al. 2021). The three main serotypes were *S*. Typhimurium (19.9%), *S*. Senftenberg (14.7%) and *S*. Agona (12.1%). Of the isolates, 293/307 (95.4%) were susceptible to all 16 tested antimicrobial agents, and all isolates were susceptible to amoxicillin-clavulanate, azithromycin, ceftiofur, ceftriaxone, ciprofloxacin, colistin, florfenicol, gentamicin, kanamycin and trimethoprim-sulfamethoxazole. There were low levels of resistance to streptomycin (2.3%), sulfisoxazole (2.0%), chloramphenicol (1.3%) and tetracycline (1.0%), ampicillin (0.7%) and cefoxitin (0.7%). Two isolates (*S*. Havana and *S*. Montevideo) were multidrug-resistant to streptomycin, sulfisoxazole and tetracycline and possessed corresponding antimicrobial resistance genes (*aadA4*, *aac(6')-Iaa*, *sul1*, *tetB*). One *S*. Typhimurium isolate was resistant to ampicillin and tetracycline, and possessed both *tetA* and *blaTEM-1B*. The absence of resistance to highest priority critically important antimicrobials among Australian commercial egg layer *Salmonella* isolates likely reflect Australia's conservative antimicrobial registration policy in food-producing animals and low rates of antimicrobial use within the industry.
- In a 2013-2014 study of *Salmonella* from eggs from Chinese farms and marketplaces, all isolates tested were resistant to sulfisoxazole (Li et al. 2020a). The majority (77.1%) of isolates were resistant to nalidixic acid, amoxicillin-clavulanate, and ampicillin, 63.9-68.9% were resistant to trimethoprim–sulfamethoxazole, kanamycin, tetracyclines, and chloramphenicol, 40.1% were resistant to ciprofloxacin, and 21.3 to 26.2% were resistant to streptomycin, ceftiofur and ceftriaxone. Resistance to gentamicin, amikacin and

cefoxitin were low (3.3–16.4%). Forty-nine (80.3%) isolates were resistant to multiple antibiotics, 32.8% of which were resistant to at least 10 antibiotics.

- In a 2016 Chinese survey of retail eggs, the predominant serotype from egg surfaces and contents was *S*. Enteritidis (64.3%); *S.* Rissen, *S.* Stanley, *S*. Tarshyne, *S*. Typhi and *S*. Virchow were also isolated (Li et al. 2020b). Of these, 65% were resistant to nalidixic acid, 39.3% to ampicillin, and 39.3% to ampicillin/sulbactam. All isolates were susceptible to ceftazidime, cefalothin, ciprofloxacin, cefepime, cefotaxime, imipenem and meropenem. Three isolates were resistant to multiple antibiotics.
- In a further survey from Chinese eggs, 97.5% of 40 *Salmonella* isolates (all *S*. Typhimurium) were multidrug-resistant (Li et al. 2022). The resistance pattern (ampicillin-colistin-streptomycin-kanamycin-gentamicin-nalidixic acid) was observed in 28 egg-sourced isolates. The colistin resistance gene *mcr-1* was frequently found in *Salmonella* isolates from retail food, especially from eggs.
- A Zambian survey that isolated *Salmonella* from eggshells at retail identified that 80% of isolates were resistant to tetracycline and 60% to ampicillin (Kapena et al. 2020).
- There were a total of three isolates from 48 eggs from Romanian producers (Tîrziu et al. 2020). The two *S*. Enteritidis isolates were resistant to azithromycin, while *S*. Infantis was also resistant ampicillin, ciprofloxacin, nalidixic acid, sulfamethoxazole; tetracycline and tigecycline.
- A total of 68.0% of *Salmonella* isolated from Polish egg samples during 2008-2012 were resistant to at least one antibiotic (Mąka et al. 2015).
- The percentage of resistant *Salmonella* isolated from table eggs in Trinidad was 22.9% (Adesiyun et al. 2007). Among isolates from that study, 14.9% were resistant to streptomycin, 6.8% to nalidixic acid, 2.7% to kanamycin and 1.4% to gentamycin.
- All isolates from eggshells and egg contents from Nigeria were susceptible to ampicillin, chloramphenicol, florfenicol, and kanamycin (Agbaje et al. 2021). However, resistance was reported for sulfamethoxazole (80% of isolates), ciprofloxacin (50% of isolates) and tetracycline (30% of isolates).

B.2 SALMONELLOSIS IN OTHER COUNTRIES

B.2.1 Adverse health effects in other countries

Table 17 shows the reported incidence of salmonellosis in a selection of other countries. Data are also shown for the most commonly observed serotypes from clinical cases in those countries or regions.

Table 17. Reported incidence data for notified cases of salmonellosis and commonly observed serotypes in other countries or regions, 2018-2022.

Country	Incidence (cases/100,000)	Year	Top 3 serotypes (% of total cases where reported, or population incidence)	Reference/source
Australia	56.3	2018	*S.* Typhimurium (32.5%), *S.* Enteritidis (6.7%), *S.* Virchow (4.6%)	Australian Department of Health and Aged Care, National Notifiable Disease Surveillance System[1]
	57.5	2019	*S.* Typhimurium (33.4%), *S.* Enteritidis (7.8%), *S.* Virchow (4.5%)	
	46.9	2020	*S.* Typhimurium (41.6%), *S.* Saintpaul (5.7%), *S.* Virchow (4.9%)	
	41.7	2021	*S.* Typhimurium (31.2%), *S.* Saintpaul (10.9%), *S.* Virchow (5.4%)	
Canada	19.7	2018	*S.* Enteritidis (42%), *S.* Typhimurium (8%), Heidelberg (5%)	(Government of Canada 2019, 2020)
	16.9	2019	*S.* Enteritidis (50.9%), *S.* Typhimurium (12.6%), monophasic *S.* Typhimurium (1,4, [5],12:i:-) (6.6%)	
European Union	20.1	2018	*S.* Enteritidis (60.9%), *S.* Typhimurium (13.8%), monophasic *S.* Typhimurium (1,4, [5],12:i:-) (4.7%)	(European Food Safety Authority and European Centre for Disease Prevention and Control 2019)
	20.0	2019	*S.* Enteritidis (50.3%), *S.* Typhimurium (11.9%), monophasic *S.* Typhimurium (1,4, [5],12:i:-) (8.2%)	(European Food Safety Authority and European Centre for Disease Prevention and Control 2021a)
	13.7	2020	*S.* Enteritidis (48.7%), *S.* Typhimurium (12.4%), monophasic *S.* Typhimurium (1,4, [5],12:i:-) (11.1%)	(European Food Safety Authority and European Centre for Disease Prevention and Control 2021b)
	15.7	2021	*S.* Enteritidis (54.6%), *S.* Typhimurium (11.4%), monophasic *S.* Typhimurium (1,4, [5],12:i:-) (8.8%)	(European Food Safety Authority and European Centre for Disease Prevention and Control 2022)
US[2]	18.3	2018	*S.* Enteritidis (2.6 per 100,000 population), *S.* Newport (1.6 per 100,000 population), *S.* Typhimurium (1.5 per 100,000 population)	(Tack et al. 2019)
	17.1	2019	*S.* Enteritidis (2.6 per 100,000 population), *S.* Newport (1.4 per 100,000 population), *S.* Typhimurium (1.3 per 100,000 population)	(Tack et al. 2020)
	13.3	2020	*S.* Enteritidis (1.6 per 100,000 population), *S.* Newport (1.5 per 100,000 population),	(Ray et al. 2021)

Country	Incidence (cases/100,000)	Year	Top 3 serotypes (% of total cases where reported, or population incidence)	Reference/source
			S. Javiana (1.0 per 100,000 population)	
	14.2	2021	*S.* Enteritidis (17%), *S.* Newport (11%), *S.* Typhimurium (9%)	(Collins et al. 2022)
New Zealand	22.5	2018	*S.* Typhimurium (34.0%), *S.* Enteritidis (12.8%), *S.* Bovismorbificans (8.1%)	See Table 6 and Table 7 [7]
	24.2	2019	*S.* Typhimurium (39.1%), *S.* Enteritidis (15.8%), *S.* Bovismorbificans (4.7%)	
	13.9	2020	*S.* Typhimurium (49.7%), *S.* Enteritidis (10.7%), *S.* Bovismorbificans (8.6%)	
	13.9	2021	Typhimurium (47.6%), Enteritidis (19.5%), Bovismorbificans (7.4%)	

[1] Australian data was extracted from the websites: https://nindss.health.gov.au/pbi-dashboard/ (salmonellosis yearly incidence) and https://www.health.gov.au/resources/publications/national-notifiable-diseases-surveillance-system-nndss-public-dataset-salmonella?language=en (*Salmonella* serotypes). Data from NNDSS was presented as total cases; the rate was calculated from the Australian Bureau of Statistics; https://www.abs.gov.au/

[2] FoodNet surveillance data are from 10 US states, representing ~15% of the US population.

B.2.2 Salmonellosis outbreaks associated with eggs in other countries

Prior to 2019, New Zealand salmonellosis outbreaks associated with eggs were caused by non-Enteritidis serotypes. Thus, the 2016 Risk Profile (Rivas et al. 2016) predominantly considered egg-associated outbreaks caused by non-Enteritidis serotypes, with a focus on those from Australia. Due to the detection of *S.* Enteritidis in New Zealand egg layer flocks, the current update considers egg-associated outbreaks caused by all *Salmonella* serotypes since 2015.

Outbreaks in Australia associated with eggs: Salmonellosis outbreaks in Australia have been most frequently associated with the consumption of raw or minimally-cooked egg products (OzFoodNet Working Group 2022). As was also discussed in the 2016 Risk Profile (Rivas et al. 2016), egg-associated outbreaks have continued to increase in Australia in recent years (Moffatt et al. 2016). The reasons for this increase are not clear but a common element of many of these outbreaks is the consumption of raw or undercooked eggs. One study demonstrated that there was an increase in salmonellosis due to the consumption of egg-based sauces, desserts containing raw or lightly-cooked eggs, and Vietnamese style sandwiches which usually contain a raw-egg butter and/or pork or chicken liver pâté (Ford et al. 2018). As such, the increase may reflect changing consumer food preferences. In addition, prior to 2018, *S.* Enteritidis had also not been detected in Australian poultry and egg-associated salmonellosis outbreaks were predominantly caused by *S.* Typhimurium (Ford et al. 2018).

At the time of this report, the most recent year for which Australian outbreak data were available was 2017, egg-associated salmonellosis outbreak details from which are listed in Table 18 (Appendix). The 2016 Risk Profile (Rivas et al. 2016) covered Australian egg-

associated outbreaks up to 2013. A summary of Australian egg-associated outbreaks from 2014 to 2017 are listed below.

- 2017: There were 49 egg-associated outbreaks (27% of all foodborne outbreaks) affecting approximately 746 people, 163 of which were hospitalised, resulting in two deaths (OzFoodNet Working Group 2022). A total of 48 of the outbreaks were caused by *S*. Typhimurium with 35 different multiple-locus variable-number tandem repeat analysis (MLVA) profiles, and one was caused by *S*. Hessarek.
- 2016: There were 35 egg-associated outbreaks (20% of all foodborne outbreaks) affecting approximately 510 people, 89 of which were hospitalised (OzFoodNet Working Group 2021a). All were caused by *S*. Typhimurium, with 29 different MLVA profiles identified. Eggs accounted for nearly all of the foodborne outbreaks caused by *S*. Typhimurium. The biggest egg-related outbreak involved 143 people following the consumption of scrambled eggs at a hotel restaurant.
- 2015: There were 51 egg-associated outbreaks (48% of all foodborne outbreaks) affecting approximately 1229 people, 156 of which were hospitalised, and resulting in no deaths (OzFoodNet Working Group 2021b). A total of 46 outbreaks were caused by *S*. Typhimurium, one by *S*. Virchow, three by *Salmonella* spp. (no serotype given), and for one, the causative agent was unknown. The biggest egg-related outbreak involved 140 people, of which 9 were hospitalised, following the consumption of a rum and raisin bread with custard that was prepared by a commercial caterer.
- 2014: There were 47 egg-associated outbreaks (37% of all foodborne outbreaks) affecting approximately 741 people, 105 of which were hospitalised, and resulting in two deaths (OzFoodNet Working Group 2021b). All were caused by *S*. Typhimurium. The biggest egg-related outbreak involved 242 people, of which 26 were hospitalised, following the consumption of mayonnaise prepared at a restaurant.

Of the egg-associated outbreaks from 2014 to 2017, the preparation setting was most commonly at restaurants followed by private residences. Commonly implicated foods were desserts made from raw eggs (for example, tiramisu, chocolate mousse and fried ice cream), raw egg-based sauces and dressings (for example, mayonnaise, aioli and hollandaise sauce), as well as breakfast egg dishes and milkshakes. The same findings were reported for the period 2015 to 2020 (New South Wales Food Authority 2022).

From May 2018 to 2019, a large egg-associated outbreak caused by *S*. Enteritidis occurred in NSW, which entailed the first *S*. Enteritidis outbreak attributed to this source in Australia (New South Wales Food Authority 2019). As at 17 June 2019, there were 235 human cases linked to this outbreak, of which 224 were confirmed by WGS.[62] The majority of the cases were from NSW, but there were also some from other Australian states, and from New Zealand (Luo et al. 2021). *S*. Enteritidis was detected at thirteen egg production facilities from NSW and one from Victoria. All properties had common transport networks (that is, people, eggs or equipment were moving between them). To limit the spread of *S*. Enteritidis and protect the health of egg consumers, various risk reduction measures were put in place including movement restrictions, farm depopulation, decontamination and education around improved

[62] https://www.foodstandards.gov.au/consumer/safety/Pages/Salmonella-Enteritidis-linked-to-eggs.aspx; accessed 27 March 2023

biosecurity measures, egg recalls (as listed in Table 20) and withdrawals where required, and issuing of consumer and industry advice.

The outbreak strain was defined as a clade B lineage that was also prevalent in Europe, and was closely related, but not directly linked, to three European isolates (Luo et al. 2021). Based on genomic comparisons with international strains, it was estimated that the Australian isolates and international isolates (from the UK) had a most recent common ancestor around 2011 (95% CI, 2008 to 2012) (Luo et al. 2021). The results suggested that the outbreak strain may have been imported into Australia around or after 2011, established locally, and then caused a large outbreak.

Outbreaks in other countries associated with eggs: Examples of salmonellosis outbreaks attributed to the consumption of eggs or egg products in Europe, North America, Asia and Africa that have occurred since 2015 are detailed in Table 19. These are only outbreaks that were reported by government websites (US CDC and Government of Canada) and in the scientific literature, since these sources usually provide the best available information on risk factors and evidence for eggs as the vehicle of infection.

The majority of the egg-associated outbreaks were caused by *S*. Enteritidis (ten outbreaks). Some of these have entailed large, multi-national outbreaks, which demonstrates how a contamination event can impact multiple regions when eggs are distributed widely from a single point of origin. An example of this occurred from September 2021 to January 2022, and involved 272 confirmed cases from six countries, and two deaths (European Centre for Disease Prevention and Control and European Food Safety Authority 2022a). Eggs from farms in Spain were implicated. The outbreak strain was genomically related to a 2019 multi-country outbreak cluster which involved 801 cases. A further example of a multi-national outbreak occurred between May 2015 and Oct 2018, involving 838 confirmed cases and 371 probable cases from 18 countries, and which contributed to four deaths (Pijnacker et al. 2019). Eggs from Poland were identified as the vehicle of infection. Another large outbreak involving at least seven countries also involved eggs from Poland (European Centre for Disease Prevention and Control and European Food Safety Authority 2016). It was not clear whether this outbreak overlapped with the outbreak reported by Pijnacker et al (2019), but it was plausible because one of the of the outbreak strains had the MLVA profile as that reported by Pijnacker et al (2019). Large outbreaks of *S*. Enteritidis have occurred in China (Zhang et al. 2021, Jiang et al. 2022). China is the world's largest producer of eggs and a major egg consumer (Yang et al. 2018).

There were also outbreaks caused by eggs or egg products contaminated with *S*. Braenderup, *S.* Oranienburg, *S*. Mbandaka, *S*. Barielly and *S*. Thompson. Of all of the outbreaks listed in Table 19, the largest occurred in multiple schools in Busan, Korea, involving 2,207 cases exposed during mass meal service (Eun et al. 2019). The implicated food was chocolate cake produced by a common supplier that had been contaminated by *S*. Thompson. The outbreak strain was also identified in egg whites used to make the cake.

EFSA also compiles statistics for salmonellosis outbreaks in the EU each year for which there was strong evidence for an association with the consumption of eggs and egg products. Detail

is shown below for the years considered in this report; for each year, *Salmonella* in 'eggs and egg products' caused the highest number of strong-evidence salmonellosis outbreaks:

- 2021: 39 outbreaks, 403 cases, 79 hospitalised (European Food Safety Authority and European Centre for Disease Prevention and Control 2022);
- 2020: 37 outbreaks (25 due to *S*. Enteritidis), 303 cases, 46 hospitalised (European Food Safety Authority and European Centre for Disease Prevention and Control 2021b);
- 2019: 98 outbreaks, 1,172 cases, 351 hospitalised, 1 death (European Food Safety Authority and European Centre for Disease Prevention and Control 2021a);
- 2018: 135 outbreaks (84 due to *S*. Enteritidis), 1,989 cases, 354 hospitalised, 2 deaths (European Food Safety Authority and European Centre for Disease Prevention and Control 2019);
- 2017: 99 outbreaks (46 due to *S*. Enteritidis), 964 cases, 224 hospitalised, 3 deaths (European Food Safety Authority and European Centre for Disease Prevention Control 2018);
- 2016: 67 outbreaks, 1,099 cases, 222 hospitalised, 4 deaths (European Food Safety Authority and European Centre for Disease Prevention and Control 2017).

Large outbreaks of salmonellosis have also occurred annually in the US, associated with backyard chicken flocks.[63] Case numbers attributed to this source have been increasing, with 252 cases in 2015 to 1,722 cases in 2021. Multiple serotypes are typically implicated each year, of which *S*. Enteritidis was identified yearly from 2015 to 2022. Producers with <3000 layer hens, such as backyard poultry producers, are not subject to the US Food and Drug Administration (FDA) Egg Safety Rule for *S*. Enteritidis control prevention (Appendix C). Illness due to backyard poultry flocks may be caused by handling of poultry or chicken meat, in addition to the consumption of eggs and egg products. *S*. Enteritidis egg-associated outbreaks have been traced to farms not regulated by the Egg Safety Rule (Stilz et al. 2022).

[63] https://www.cdc.gov/healthypets/outbreaks.html#live-poultry; accessed 11 April 2023

Table 18. Examples of outbreaks of salmonellosis from the consumption of eggs and egg products in Australia (2017).[1]

Month	*Salmonella* serotype	Cases, hospitalisations, deaths[2]	Suspected food/source	Setting
Jan	Typhimurium, MLVA 03-17-09-12-523, MLVA 03-26-13-08-523	119 cases, 20 hospitalisations	Multiple foods contaminated with raw eggs	Restaurant
Jan	Typhimurium, MLVA 03-17-09-12-523	9 cases, 2 hospitalisations	French toast made with raw eggs	Picnic
Jan	Typhimurium, MLVA 03-16-09-07-523	17 cases, unknown hospitalisations	Multiple foods containing eggs or contaminated by eggs	Restaurant
Jan	Typhimurium, MLVA 03-12-10-10-523	48 cases, 16 hospitalisations	Fried ice cream	Restaurant
Jan	Typhimurium, MLVA 03-12-11-11/12-523	13 cases, 4 hospitalisations	Vietnamese rolls with raw egg aioli	Restaurant
Jan	Typhimurium, MLVA 03-15-06-11-550	6 cases, 1 hospitalisation	Multiple breakfast dishes	Restaurant
Jan	Typhimurium, MLVA 03-14-10-10-523	6 cases, 2 hospitalisations	Multiple foods including aioli containing raw eggs	Restaurant
Jan	Typhimurium, MLVA 03-14-09-11-523	4 cases	Multiple foods including raw egg sauces	Restaurant
Jan	Typhimurium, MLVA 03-13-10-09-523	11 cases, 2 hospitalisations	Egg, lettuce and pesto sandwiches	Commercial caterer
Jan	Typhimurium, MLVA 03-17-09-12-523	2 cases	Ice cream made with raw eggs	Correctional facility
Jan	Typhimurium, MLVA 03-09-09-14-523	19 cases	Vietnamese rolls with raw egg butter	Bakery
Jan	Typhimurium, MLVA 03-25-16-12-523	6 cases, 1 hospitalisation	Breakfast egg dishes	Restaurant
Jan	Typhimurium, MLVA 03-26-16-11-523	3 cases	Hollandaise sauce containing raw eggs	Restaurant
Feb	Typhimurium, MLVA 03-17-09-12-523	5 cases, 3 hospitalisations	Arancini balls bound with raw egg	Private residence
Feb	Typhimurium, MLVA 03-14-09-11-523	9 cases, 1 hospitalisation	Multiple foods contaminated with raw eggs	Restaurant
Feb	Typhimurium, MLVA 03-14-09-11-523	14 cases, 6 hospitalisations	Pies with post-cook raw egg wash	Bakery
Mar	Typhimurium, MLVA 03-20-09-12-523, MLVA 03-17-09-12-523	62 cases, 5 hospitalisations	Boiled eggs	Mining camp
Mar	Typhimurium, MLVA 03-17-09-12-523	7 cases, 2 hospitalisations	Raw egg mayonnaise/aioli	Restaurant
Mar	Typhimurium, MLVA 03-09-07-12-523	2 cases, 2 hospitalisations	Caesar salad dressing containing raw eggs	Restaurant
Mar	Typhimurium, MLVA 03-15-12-11-523	2 cases, 2 hospitalisations	Fried ice cream	Restaurant
Mar	Hessarek	27 cases, 11 hospitalisations	Eggs (Food handler contamination, inadequate washing of food eaten uncooked)	Primary production
Mar	Typhimurium, MLVA 03-16-09-12-523	13 cases, 3 hospitalisations, 1 death	Multiple foods contaminated with raw eggs	Aged care facility
Mar	Typhimurium, MLVA 03-24-12-11-523	31 cases, 17 hospitalisations	Hollandaise sauce containing raw eggs	Restaurant
Mar	Typhimurium, MLVA 03-14-08-13-523	4 cases, 1 hospitalisation	Vietnamese rolls with raw egg butter	Takeaway
Apr	Typhimurium, MLVA 03-26-13-08-523	4 cases, 3 hospitalisations	Ice cream made with raw eggs	Correctional facility
May	Typhimurium, MLVA 03-09-09-14-523	22 cases, 7 hospitalisations	Raw egg sauces	Restaurant
May	Typhimurium, MLVA 03-11-07-11-523	10 cases, 6 hospitalisations	Salmon patties bound with raw eggs	Restaurant
May	Typhimurium, MLVA 03-27-16-11-523	5 cases, 1 hospitalisation	Chocolate mousse containing raw eggs	Private residence
May	Typhimurium, MLVA 03-23-12-10-523	24 cases, 2 hospitalisations	Raw cake mixture	School

Month	*Salmonella* serotype	Cases, hospitalisations, deaths[2]	Suspected food/source	Setting
May	Typhimurium, MLVA 03-12-11-10-523	7 cases	Chocolate mousse cake containing raw eggs	Private residence
May	Typhimurium, MLVA 03-25-16-11-523	29 cases, 2 hospitalisations	Egg casserole	Childcare centre
May	Typhimurium, MLVA 03-17-09-12-523	5 cases, 4 hospitalisations	Fresh pasta containing raw eggs	Private residence
Jun	Typhimurium, MLVA 03-22-14-11-523	6 cases	Arancini balls bound with raw egg	Restaurant
Jun	Typhimurium, MLVA 03-17-09-12-523	13 cases, 3 hospitalisations	Vietnamese rolls with raw egg butter	Restaurant
Jul	Typhimurium, MLVA 03-14-09-11-523	15 cases, 1 hospitalisation	Pikelets	Childcare centre
Jul	Typhimurium, MLVA 03-17-10-12-523	3 cases, 3 hospitalisations	Raw muffin batter containing raw eggs	Private residence
Aug	Typhimurium, MLVA 03-17-09-12-523	3 cases	Hamburger patties bound with raw eggs	Restaurant
Sep	Typhimurium, MLVA 03-10-15/16-11-496	4 cases, 1 hospitalisation	Chocolate mousse containing raw eggs	Private residence
Sep	Typhimurium, MLVA 03-12-11-10-523	4 cases, 1 hospitalisation	Chocolate soufflé containing raw eggs	Restaurant
Oct	Typhimurium, MLVA 03-26-16-12-523	10 cases, 1 hospitalisation	Breakfast egg dishes	Restaurant
Oct	Typhimurium, MLVA 03-12-10-11-523	9 cases	Meat cannelloni containing eggs	Private residence
Oct	Typhimurium, MLVA 03-22-13-11-523	4 cases, 2 hospitalisations	Multiple foods contaminated with raw eggs	Private restaurant
Oct	Typhimurium, MLVA 03-22-13-11-523	9 cases, 2 hospitalisations	Fried ice cream	Restaurant
Oct	Typhimurium, MLVA 03-12-11-10-523	8 cases, 1 hospitalisation	Multiple foods containing eggs	Restaurant
Nov	Typhimurium, MLVA 03-15-10-08-523	8 cases, 3 hospitalisations	Salad with raw egg mayonnaise	Private residence
Dec	Typhimurium, MLVA 03-14-11-08-523	73 cases, 13 hospitalisations, 1 death	Sandwiches/wraps/ rolls containing chicken/ contaminated by eggs	Bakery
Dec	Typhimurium, MLVA 03-13-11-12-496	9 cases, 2 hospitalisations	Raw egg mayonnaise	Restaurant
Dec	Typhimurium, MLVA 03-17-09-11/12-523	8 cases	Arancini made with raw egg mayonnaise or tiramisu	Restaurant
Dec	Typhimurium, MLVA 03-17-07-12-523	15 cases, 4 hospitalisations	Vietnamese rolls with raw egg butter	Restaurant

[1] Data source: (OzFoodNet Working Group 2022).

[2] Data was only listed for deaths or hospitalisations when there was at least one occurrence.

Table 19. Examples of outbreaks of salmonellosis from the consumption of eggs and egg products in countries other than Australia and New Zealand (studies published from 2016 to February 2023).

Country	Year	*Salmonella* serotype	Cases, hospitalisations, deaths	Suspected food/source	Reference/data source
North America					
Canada	2020-2021	Enteritidis	70 cases, 19 hospitalisations, 0 deaths	Eggs that had been recalled	https://www.canada.ca/en/public-health/services/public-health-notices/2021/outbreak-salmonella-infections-eggs.html
US	2018	Enteritidis	44 cases, 12 hospitalisations, 0 deaths	Eggs (epidemiological, traceback and laboratory evidence; outbreak strain detected in poultry environment and in eggs)	https://www.cdc.gov/salmonella/enteritidis-09-18/index.html
US	2018	Braenderup	45 cases, 11 hospitalisations, 0 deaths	Eggs (epidemiological, traceback and laboratory evidence; outbreak strain detected in poultry environment)	https://www.cdc.gov/salmonella/braenderup-04-18/index.html
US	2016	Oranienburg	8 cases, 2 hospitalisations, 0 deaths	Eggs (epidemiological, trace-back and laboratory evidence; outbreak strain detected in poultry environment and in eggs)	https://www.cdc.gov/salmonella/oranienburg-10-16/index.html
Europe					
France, UK, Denmark, Netherlands, Norway, Spain; originating in Spain	2020-2021	Enteritidis ST11	272 cases, 25 hospitalisations, 2 deaths; 801 historical outbreak-related cases also identified by WGS	Eggs (epidemiological, trace-back investigations, detection from egg layer environment and eggshell)	(European Centre for Disease Prevention and Control and European Food Safety Authority 2022a)
18 countries, originating in Poland	2015-2018	Enteritidis MLVA 2-9-6-3-2	838 confirmed and 371 probable cases, 89/246 (36%) hospitalisations, 4 deaths	Eggs (case-control study, epidemiological evidence, trace-back, trace-forward, and environmental investigations)	(Pijnacker et al. 2019)

Country	Year	*Salmonella* serotype	Cases, hospitalisations, deaths	Suspected food/source	Reference/data source
Belgium, Denmark, Luxembourg, Netherlands, Norway, Sweden, UK, and likely also Croatia; originating in Poland	2016	Enteritidis DT8, MLVA 2-9-6-3-2, MLVA 2-9-7-3-2	112 confirmed and 148 probable cases, hospitalisations not reported, 1 death with epidemiological link (Croatia)	Eggs (trace-back investigations, detection of strains in and on eggs)	(European Centre for Disease Prevention and Control and European Food Safety Authority 2016)
Serbia	2018	Mbandaka	8 cases, 0 hospitalisations, 0 deaths	Eggs	(European Centre for Disease Prevention and Control and European Food Safety Authority 2022b)
Czech Republic, Slovakia	2017-2018	Bareilly	325 cases, 92/299 (30.8%) hospitalisations, 0 deaths	Egg products (powdered egg product caused by cross-contamination from spray dryer, not clear if eggs were original source. Epidemiological evidence, outbreak strain identified in egg product and spray dryer)	(Labská et al. 2021)
UK, Spain	2015	Enteritidis	154 cases, 26 (19·1%) hospitalisations, 0 deaths	Eggs (epidemiological evidence, trace-back investigations, outbreak strain detected in egg product, food handler and food surface; genomically related strain identified in egg distribution network)	(Inns et al. 2017)
Asia					
China	2021	Enteritidis	225 cases; hospitalisations and deaths not reported	Eggs (egg fried rice; epidemiological evidence, trace-back investigations, outbreak strain detected in egg product)	(Zhang et al. 2021)

Country	Year	*Salmonella* serotype	Cases, hospitalisations, deaths	Suspected food/source	Reference/data source
China	2019	Enteritidis	157 cases, 1 hospitalisation, 0 deaths	Eggs (egg sandwiches with kitchen-made mayonnaise; epidemiological evidence, trace-back investigations, outbreak strain detected in eggs and egg products)	(Jiang et al. 2022)
Taiwan	2018	Enteritidis	47 cases, 14 hospitalisations, 1 death	Eggs (French toast sandwiches; epidemiological evidence, surveillance footage of eggshell contamination of egg product and inadequate food handling)	(Chueh et al. 2020)
South Korea	2018	Thompson	2,207 cases, 151/1111 (13.6%) hospitalisations, 0 deaths	Eggs (chocolate cake; epidemiological evidence, trace-back investigations, outbreak strain detected in egg whites, product, cookware)	(Eun et al. 2019)
Other countries					
South Africa	2018	Enteritidis (2 outbreaks with highly related strain)	Outbreak 1: 27 cases Outbreak 2: 16 cases Hospitalisations and deaths not reported	Eggs (eggs and hollandaise sauce; epidemiological evidence, outbreak strain detected in raw eggs and egg product)	(Smith et al. 2020)

[1] Based on commonalities between these outbreaks such as MLVA type, date and country range, these may be the same outbreak.

B.2.3 Recalls from other countries

This section provides a summary of recalls of eggs and egg products for potential contamination with *Salmonella* from Australia, Canada, the EU, the UK and the US. Recalls are not necessarily linked to human illness. It was necessary to take different approaches with each recall database since these operate in different ways. The sources and methods used to retrieve the recall data were as follows:

- Australia: Food recalls recorded by Food Standards Australia New Zealand (FSANZ)[64] were scanned for relevant records using the keyword "Salmonella". Recalls were only listed back to June 2019.
- Canada: All recalls reported by the Canadian Food Inspection Agency[65] were scanned for relevant records using the keyword "egg" and filter "Food recall warning". The Government of Canada site[66] was also searched for "egg recall Salmonella".
- EU: A search function[67] was used to retrieve records from the Rapid Alert System for Food and Feed, from 1 January 2015. There are 31 countries that participate in this system. Search categories included: Countries: "Any"; Notification type: "food", notification classification: "alert"; Product category: "eggs and egg products", Hazard category: "pathogenic micro-organisms". Note that only data from 2020 onwards are currently available.
- UK: All recalls reported by the UK Food Standards Agency[68] from 1 January 2015 to February 2023 were examined for relevant records; no relevant records were identified prior to December 2020.
- US: All recalls reported by the US Department of Agriculture Food Safety and Inspection Service[69] from 1 January 2015 to February 2023 were scanned using the search term "Salmonella" for relevant records. CDC outbreak reports[70] were also searched.

Recalls of eggs and egg products due to the potential for *Salmonella* contamination for the period 2015 to February 2023 are listed in Table 20. There were no UK egg or egg product recalls identified. There were two recalls each from Canada and Australia, three from the US and 19 recalls from the EU. The recall of Australian eggs due to *S*. Enteritidis involved multiple producers. Where the serotype was listed, it most often included *S*. Enteritidis (13 listings), as well as one recall each due to Group D *Salmonella* (this serogroup includes *S*. Enteritidis), *S*. Mbandaka, *S*. Braenderup and *S*. Oranienburg. Recalls were most often due to the detection of *Salmonella* in or on eggs or egg products; detection in the layer environment was also listed. Seven recalls were reported to be associated with a possible or confirmed association with human cases of salmonellosis. Sixteen of the recalls were of eggs, five were of whole egg or yolk powder, three were of egg whites, one was of egg yolk, one of scrambled egg mix and a final recall was from bakery products made from eggs in addition to the eggs.

[64] http://www.foodstandards.gov.au; accessed 5 March 2023
[65] https://recalls-rappels.canada.ca/en; accessed 5 March 2023
[66] https://www.canada.ca/en.html; accessed 5 March 2023
[67] https://webgate.ec.europa.eu/rasff-window/screen/search; accessed 5 March 2023
[68] https://www.food.gov.uk/search?keywords=&filter_type%5BFood%20alert%5D=Food%20alert; accessed 5 March 2023
[69] https://www.fsis.usda.gov/recalls; accessed 5 March 2023
[70] https://www.cdc.gov/salmonella/outbreaks-active.html; accessed 6 March 2023

Table 20. Recalls of eggs destined for human consumption due to detected or suspected *Salmonella* contamination: Australia[1], Canada[2] and the EU[3] (January 2015-February 2022).

Country of recall/ notification	Country of food origin	Date of recall	Product	Serotype or concern (where reported)
Australia	Australia	21 Jan 2023	Eggs (possible association with salmonellosis case)	
Australia	Australia	8 Sep 2018-14 Jun 2019	Eggs from multiple farms (detection from layer farms of rare strain in Australian poultry found to be associated with human illness due to egg consumption)	Enteritidis
Canada	Canada	21 Nov 2020	Eggs (detection during testing; not indicated whether eggs or layer environment)	
Canada	Canada	8 Oct 2020 12 Dec 2020	Eggs (detection during testing; not indicated whether eggs or layer environment); bakery products made from this brand	
US	US	8 Sep 2018	Eggs (traceback during salmonellosis outbreak investigation, detection in layer environment samples and in eggs)	Enteritidis
US	US	13-16 Apr 2018	Eggs (traceback during salmonellosis outbreak investigation, detection in layer environment samples)	Braenderup
US	US	3 Oct 2016	Eggs (traceback during salmonellosis outbreak investigation, detection in layer environment samples)	Oranienburg
Netherlands	Netherlands	13 Jan 2023	Chicken eggs (detection in laying hens)	Enteritidis
Sweden	Sweden	10 Jan 2023	Eggs (detection in egg; suspected case following consumption of eggs from company)	
Spain	France	31 Aug 2022	Pasteurised egg whites (detection from product)	
Poland	Bulgaria	31 May 2022	Whole egg powder (detection from product)	Enteritidis
Poland	Bulgaria	6 May 2022	Whole egg powder (detection from product)	Enteritidis
Lithuania	Bulgaria	6 May 2022	Whole egg powder (detection from product)	Enteritidis
Poland	Poland	9 Mar 2022	Eggs (detection from product)	Enteritidis
Poland	Poland	4 Nov 2021	Eggs (detection in environmental samples)	Enteritidis
Denmark	Denmark	1 Nov 2021	Eggs (associated with food outbreak)	Enteritidis
Poland	Poland	13 Sep 2021	Eggs (detection on eggshells and contents)	Group D
Poland	Poland	18 Jun 2021	Eggs (detection on eggshells)	Enteritidis
Sweden	Poland	13 Jan 2021	Egg yolk powder (detection)	Mbandaka
Belgium		3 Dec 2020	Pasteurised egg yolk (detection)	
Poland	Poland	1 Sep 2020	Eggs (possible detection on eggshell)	Enteritidis
Latvia	Ukraine	20 Aug 2020	Scrambled eggs mix (detection in product)	Enteritidis
Poland	Bulgaria	19 Aug 2020	Egg powder (detection in product)	Enteritidis
Italy	Poland	16 Jul 2020	Eggs (detection in eggs)	
Denmark	Netherlands	11 Jun 2020	Chicken egg whites (detection)	
Belgium	Belgium	14 May 2020	Liquid egg white (detection)	

[1] http://www.foodstandards.gov.au; accessed 5 March 2023

[2] https://www.canada.ca/en.html; accessed 5 March 2023

[3] https://webgate.ec.europa.eu/rasff-window/screen/search; accessed 5 March 2023

B.2.4 Risk assessments and risk-related activities overseas

Guidance for undertaking formal risk assessments has been produced by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO),[71] and by the USDA and US Environmental Protection Agency (United States Department of Agriculture Food Safety and Inspection Service and United States Environmental Protection Agency 2012). In addition, there have been several models published that estimate the probability of salmonellosis through exposure to egg products.[72]

Australian Risk Assessments

An update has been provided of an earlier 2013 risk assessment by the NSW Food Authority egg food safety scheme (New South Wales Food Authority 2022). A key focus of the assessment was on interventions for *S*. Enteritidis, which had been detected in Australian layer flocks since the 2013 risk assessment. The authors state that as *S*. Enteritidis can be highly persistent in both infected birds and diverse environmental reservoirs, complete eradication from the Australian egg production environment is challenging. Instead, it is prudent to view *S*. Enteritidis as part of the broad ecosystem and therefore a continuing threat to commercial egg production.

While the risk characterisation largely focussed on the control of S. Enteritidis, the report noted that *S*. Typhimurium was responsible for the majority (83%) of all egg-related outbreaks from 2013 to 2020. However, the number of cases of foodborne salmonellosis involving *S*. Typhimurium declined by 65% from 2014 to 2018, which was attributed to controls under the NSW Food Safety Strategy 2015–2021 and largescale vaccination of layer flocks against this serotype. Because restaurants, bakeries, private residences and take-away venues were most commonly implicated in these outbreaks, the report recommended further promotion of educational material already prepared by the NSW Food Authority, such as guidance on risky egg handling practices and the safe preparation of raw egg products.

EU

A 2019 Scientific Opinion investigating contributory factors and control options in poultry production was produced by EFSA in response to an increase of human salmonellosis in the EU after 2014 (European Food Safety Authority Panel on Biological Hazards 2019). A qualitative assessment considered the public health impact if target serotypes of *Salmonella* in breeding flocks were changed (currently, *S*. Enteritidis, *S*. Typhimurium, *S*. Hadar, *S*. Virchow and *S*. Infantis). There was deemed justification for retaining *S*. Enteritidis, *S*. Typhimurium and *S*. Infantis as targets in breeding flocks. *S*. Kentucky was proposed as a target serotype because it had spread among poultry in the EU, and because many strains were resistant to antimicrobials, including fluoroquinolones. Other options included *S*. Heidelberg (based on increased potential for vertical transmission and antimicrobial resistance), *S*. Thompson based on its occurrence in breeding flocks, or a variable serotype based on the member state.

[71] https://www.fao.org/food-safety/food-control-systems/risk-and-evidence-base/risk-based-approaches-and-tools/en/; accessed 30 June 2023
[72] https://www.foodrisk.org/; accessed 30 May 2023

The impact of a 1% target of "all *Salmonella* serotypes" in breeding flocks was also considered. However, this target was deemed difficult to achieve, and under this scenario 18 of the 24 Member States would have failed the target at some point during 2014 to 2016. This could result in unnecessary control action and loss of sources of food if more breeding flocks are culled. Benefits would include a greater emphasis on the feed industry because certain serotypes were likely to be introduced by feed. Reporting all serotypes would also provide data for source attribution and epidemiological studies.

The assessment also estimated the impact on human salmonellosis cases if the target set for adult flocks of laying hens was reduced from 2% to 1% for the current target serotypes (*S.* Enteritidis and *S.* Typhimurium, including monophasic variants), and maintaining the current testing scheme and trade restrictions. For 2016, seven Member States failed the 2% prevalence target for target serotypes; while 15 Member States would have failed a 1% target. This target was estimated to result in a 53% (95% Credibility Interval: 39%; 66%) reduction in human salmonellosis cases (based on 2016 data).

A further focus of the assessment was on risk factors for the occurrence of *Salmonella* in relation to farming management, housing systems and biosecurity variables for layer chickens. As discussed elsewhere in this Risk Profile, the study found that overall evidence pointed to a lower occurrence of *Salmonella* for layer hens in cage-free compared with caged housing systems.

A quantitative risk assessment model has also been produced to estimate the number of salmonellosis cases in the EU per million servings of table eggs, as well as the probability of illness when ingesting a random serving of table egg (Desvignes et al. 2019). The model took into account different parameters along the farm-to-consumer chain that would influence the potential for contamination and growth of *S*. Enteritidis in eggs, including storage time. It also considered consumer practices and cooking times and temperatures. As an example of an output of the model, when 10% was entered for egg prevalence (which is considerably higher than actual prevalence would be), the estimated number of salmonellosis cases would be 186 salmonellosis per million serving of lightly-cooked eggs. The risk levels of well-cooked eggs were 1,690 lower than lightly-cooked eggs. The model can also be adapted for more realistic parameter settings.

Additional risk assessments have been conducted regarding consumer handling practices relating to egg safety, and egg storage (Fikiin et al. 2020, Cardoso et al. 2021, Junqueira et al. 2022). These have been discussed elsewhere in this report (Section 5.2).

US

For egg contents contaminated with *S*. Enteritidis, modelling has estimated that for eggs stored 5 days at 18˚C (following 36 h at 24˚C in the layer house), the mean numbers of *S*. Enteritidis internal contamination are 30-fold higher than for eggs stored at 7˚C (Pouillot et al. 2020). The increased levels of contamination lead to a 47-fold increase in salmonellosis risk from consumption of egg products made from these eggs (with some variation in the public health risk on the basis of the egg product type). Assuming that 7% of the liquid egg product supply originated from eggs stored at 18˚C versus 7˚C, the study estimated an additional burden of 3,562 cases of salmonellosis per year in the US.

APPENDIX C: CONTROL MEASURES

C.1 ENVIRONMENTAL TESTING FOR *S.* ENTERITIDIS IN NEW ZEALAND BREEDER AND LAYER FLOCKS

Routine environmental sampling of New Zealand poultry for *S.* Enteritidis came into effect on 6 October 2022 as part of the *S.* Enteritidis management framework. Sampling schedules for *S.* Enteritidis specific to each layer industry facility type include (Ministry for Primary Industries 2022c):

- Breeder flocks: sampling of every flock, in each breeder shed, every five weeks while populated.
- Hatcheries: post-hatch testing of hatcher paper, hatcher tray swabs or fluff for each hatcher completing hatching on each hatcher day (unless the business owns its supply chain); and sampling of drains plus 5 additional sample types every hatch week (or every 2 weeks while the hatcher is idle).
- Rearer farms: sampling of all populated production areas when chickens are 2-5 weeks, and 12-18 weeks (and at a time that ensures the results are received before the flock enters a laying production area).
- Layer farms: all production areas containing single-age flocks at approximately the mid-lay stage of the flock, or every 20 weeks for production areas containing multi-age flocks.

The number of each sample type and for each facility type are listed in Table 21.

Table 21. Numbers and types of samples collected during routine environmental sampling for *S.* Enteritidis of the different egg layer industry facility types.[1]

Facility	Sample type			Total samples
	Pooled dust / dust swab	Faeces/ boot swabs	Other	
Breeder farm	2 (drinkers, feeders, ventilation ducting, beams, ledges)	4 boot swabs or 8 manure belt swabs	NA	6 or 10; samples may be combined and tested as single sample
Hatchery	NA	NA	Per hatch day: Hatcher paper, hatch tray swabs or chick fluff (Does not specify number)	1 composite samples may be combined and tested as single sample
	NA	NA	Per hatch week: Swabs from macerator, meconium, drain, egg loading room, transfer room, pull belt, air handling units (dust), air transfer machine (dust) chick take-off and carousel), dead chicks, and/or wastewater samples (x5)	5
Rearer farm	2 (drinkers, feeders, ventilation ducting, beams, ledges)	4 boot swabs or 8 manure belt swabs	NA	6 or 10; samples may be combined and tested as single sample
Layer farm	2 (drinkers, feeders, ventilation ducting, beams, ledges)	4 boot swabs or 8 manure belt swabs	NA	6 or 10; samples may be combined and tested as single sample

[1] Data source: (Ministry for Primary Industries 2022c)

Laboratory testing of environmental samples is only required to report on the presence/absence of *S*. Enteritidis; there are no requirements for reporting other *Salmonella* serotypes. An *S*. Enteritidis positive result may either consist of a sample with an isolate serotyped as *Salmonella* Group D (O:9, H:g,m): b) or a sample positive for *S*. Enteritidis by PCR screen. A *S*. Enteritidis negative result may either consist of a "not detected" result by PCR screen for *Salmonella* spp. or *S.* Enteritidis, a sample without colonies on selective agar plates morphologically typical of *Salmonella* spp., or a sample with isolates negative by serotyping for *Salmonella* Group D.

Following an *S*. Enteritidis detection, laboratories must report results to the poultry producer and MPI Director-General within 24 hours. The poultry producer must then categorise and isolate all affected areas, chickens and eggs within those areas as *S*. Enteritidis-positive and notify the verifier and every producer or processor immediately before and after them in the supply chain. This includes every person from whom they may have received potentially contaminated chickens or eggs, or to whom they have supplied potentially contaminated chickens or eggs. Within 48 hours, the producer must provide a report to the MPI Director-General with information such as the locations of *S*. Enteritidis detection, an inventory of chickens produced since the positive detection and their location, and a summary of controls and investigation details. *S*. Enteritidis-positive flocks and eggs must be disposed of in a manner by which they do not contaminate other animals, product, production areas, or the environment, and do not enter the human food chain. Alternatively, the eggs may be treated in a manner that has been validated to reduce any *S*. Enteritidis present (for example, pasteurisation), and that does not contaminate other eggs, the processing environment or equipment. Such eggs may then be supplied to a processor for human or animal consumption.

In the event of a false-positive result, *S*. Enteritidis-false positive production areas can be returned to negative following *S*. Enteritidis-negative test results from intensive testing consisting of enhanced environmental sampling and animal material from 100 euthanised chickens (cloacal swabs, whole tissues samples from the caecum, and for hens, whole periovarian tissue (ovaries and oviduct)). Alternatively, returning positive areas to negative requires *S*. Enteritidis-negative environmental test results after depopulation and extensive cleaning and sanitation. Due to the greater risk of dissemination posed by breeder chickens and hatcheries, more frequent, enhanced environmental testing is required. For breeders, this includes three sampling rounds at one week after repopulation, with at least five days in between rounds, and additional sample numbers. For hatcheries, this includes every hatch day until three consecutive negative *S*. Enteritidis results are obtained.

The environmental sampling requirements and testing frequency for farms that export eggs differ from the routine testing of breeder and layer flocks. Sample numbers and types for different shed lengths and housing systems are shown in Table 22; rodent droppings and swabs of rodents from traps may also be conducted. Testing frequency includes:

- Rearer layer flocks: testing in the two weeks before the flock is moved to the laying shed, or before the estimated point of lay for birds that remain in the same shed for laying;
- Layer flocks in lay: testing in the first week of lay, then within five weeks later, and thereafter at least three-monthly during the laying period.

Table 22. Numbers and types of samples collected based on layer shed length and system during environmental sampling of exporting layer farms for *S.* Enteritidis.[1]

Shed length	Barn/aviary/free range		Conventional/colony	
	Faeces/boot swabs	Pooled dust /dust swab	Faeces/boot swabs	Pooled dust/dust swab
Up to 25 m	1 pair boot swabs	2 dust swabs or 2 x 25 g pooled dust samples	One manure belt boot swab per row or from full run of cross-conveyor on multi-tier rows if present (the latter preferred)	2 dust swabs or 2 x 25 g pooled dust samples
25-75 m	2 pair boot swabs	4 dust swabs or 4 x 25 g pooled dust samples		4 dust swabs or 4 x 25 g pooled dust samples
>75 m	4 pair boot swabs	8 dust swabs or 8 x 25 g pooled dust samples		8 dust swabs or 8 x 25 g pooled dust samples

[1] Data source: (Ministry for Primary Industries 2022e)

All isolates of *S*. Enteritidis, or of *Salmonella* where *S*. Enteritidis cannot be excluded, must be forwarded to ESR for further investigation and serotyping (if necessary). Note that forwarding of confirmed isolates of *S*. Enteritidis is not a requirement for routine testing of environmental samples described in the previous section.

C.2 CONTROL MEASURES IN OTHER COUNTRIES

C.2.1 Australia

Prior to 2019, there was no required routine environmental testing for *Salmonella* conducted on Australian egg layer farms. Like New Zealand, *S*. Enteritidis was previously not considered to be endemic in Australian poultry flocks (Chousalkar et al. 2018b). Australia (NSW and Victoria) has a voluntary National *Salmonella* Enteritidis Monitoring and Accreditation Program (NSEMAP) available for commercial egg producers throughout Australia.[73] This was used in particular as evidence of the *S*. Enteritidis-free status of these flocks and their eggs for export markets. Detection of *S*. Enteritidis in poultry is notifiable in Australia. Although there had been no formalised government response programme if *S*. Enteritidis was detected, Australian Eggs had produced response plan guidelines.[74]

Following the 2018-2019 *S*. Enteritidis outbreak that was associated with eggs and egg layer farms (Appendix B.2.2), the NSW Government introduced the Biosecurity (*S.* Enteritidis) Control Order 2019 to assist in the management of the biosecurity risk posed by the spread of *S.* Enteritidis. The legally enforceable order established minimum biosecurity standards for the poultry and egg industries. Under a revised control order introduced in mid-2020, licensed egg producers in NSW are also required to regularly test for *S.* Enteritidis (Biosecurity & Food Safety New South Wales Department of Primary Industries 2020, New South Wales Department of Primary Industries 2020). The Control Order is in effect until the 30th of June 2024.

For mandatory environmental testing, NSW licenced egg producers must either join NSEMAP or undertake sampling of every shed every 12-15 weeks as required by NSW Department of Primary Industries. Both approaches require sending samples to a NSEMAP accredited

[73] https://www.dpi.nsw.gov.au/animals-and-livestock/poultry-and-birds/health-disease/national-salmonella-enteritidis-monitoring-and-accreditation-program; accessed 17 January 2023
[74] https://www.australianeggs.org.au/search?q=enteritidis+response+plan; accessed 17 January 2023

laboratory. Sample types include drag swabs over rows or manure belts of the laying sheds. The areas swabbed and number of swabs per shed depends on the housing system, and may include from two to four swabs per shed.

As discussed in the 2016 Risk Profile (Rivas et al. 2016), the Primary Production and Processing Standard 4.2.5 for Eggs and Egg Products was gazetted by FSANZ in May 2011. Amendments have since been added and the current version of the Standard commenced on 29 November 2018.[75] This Standard (which applies to Australia and not New Zealand) was developed in response to the foodborne illness outbreaks suspected of being linked to eggs or egg products, particularly cracked and dirty eggs.

Overall, the standard aims to reduce the incidence of illness associated with eggs (especially cracked and dirty eggs) and egg products, particularly uncooked or lightly-cooked foods containing contaminated raw egg (for example, sauces and desserts) by:

- legally requiring egg producers and processors to identify and control safety hazards, such as ensuring feed is not contaminated;
- prohibiting the sale of cracked and dirty eggs unless they are sold to a processor for pasteurisation; and
- requiring individual eggs to be stamped with the producers' unique identification so they can be traced.

The Government of each Australian State or Territory is responsible for preparing specific regulations to enable compliance with the Standard and these are known as Food Safety Schemes for egg and egg product industries, which essentially require primary producers of eggs to be licensed and to implement food safety programs which are inspected and audited by the State or Territorial Authority (New South Wales Food Authority 2022). Implementation of these Schemes is expected to improve egg handling and processing practices resulting in production of safer and cleaner eggs by businesses.

A '*Salmonella* Initiative' was introduced by the Australian Egg Corporation Ltd. in September 2014, as well as a *Salmonella* risk assessment toolkit.[76,77] The primary aim of the initiative is to collate readily available information regarding through-chain *Salmonella* risk management, and make it more accessible to the entire egg industry and other stakeholders.

Salmonella vaccination of poultry is not mandatory in Australia, but a majority of flocks are vaccinated (75% in 2017-2019). Currently in Australia there are two registered live *S*. Typhimurium vaccines that are marketed as an aid in the control of *Salmonella* (Vaxsafe ST (Bioproperties®) and Poulvac ST (Zoetis®)) (New South Wales Food Authority 2022). Both vaccines are based on attenuated strains with a disruption of the *aroA* gene (involved in aromatic amino acid biosynthesis).

Although there are no requirements for egg storage temperature in Australia, Australian Eggs recommends that eggs are stored at temperatures of below 15˚C as soon as possible after

[75] https://www.legislation.gov.au/Details/F2018C00937; accessed 12 April 2023
[76] https://www.australianeggs.org.au/for-farmers/resources/food-safety; accessed 13 April 2023
[77] https://www.australianeggs.org.au/for-farmers/tools-and-training/salmonella-risk-assessment-toolkit; accessed 13 April 2023

collection and washed within four days of being laid.[78] Regulation of humidity in cool storage rooms is also recommended to limit condensation forming on eggs; humidity levels around 60-70% are considered appropriate. They also recommend storing eggs at less than 4˚C for end-users, especially if the eggs will be used in raw or low-cooked product.

C.2.2 Canada

There are standards for Canadian hatcheries and supply flocks for testing of all *Salmonella.*[79] For hatcheries, samples of fluff or chick box liners must be collected every sixth week while the hatchery is in operation (from all hatchers if there are less than four hatches a week, or on two consecutive days if there are four or more hatches in a week). Embryonated eggs must also been sampled within six weeks of egg setting and at the end of incubation (at least six samples from each supply). All hatcheries and breeding flocks must be free from *S*. Pullorum and *S*. Gallinarum; testing also includes testing dead, frozen or cull breeding flock birds as well as sero-testing. Primary breeding supply flocks and hatcheries must also be negative for *S*. Enteritidis ("*S*. Enteritidis-clean"). For primary breeding supply flocks, if it cannot be verified that the supply flock originated from a source verified to be *S*. Enteritidis-clean, then fluff samples (or chick box liners) must be tested for all *Salmonella* and must be negative for *S.* Enteritidis. Environmental samples must be collected from the supply flock. A minimum of one pair (or two pieces) of bootie (pooled samples) or drag swabs (pooled samples) and one dust swab in the barn for every 300-5000 birds must be tested. The number of samples must increase for every 5000-bird increments.

The Canadian Government has also produced guidance on reducing the risk of *S*. Enteritidis in shell eggs (Bureau of Microbial Hazards 2013). At the time of the document, egg producers with regulated layer flocks supplying eggs for the table egg market must perform environmental testing for *S*. Enteritidis. The minimum sampling protocol for environmental testing specifies the following:

- Mandatory *S*. Enteritidis environmental testing of layer flock at least twice during the laying cycle;
- Sampling carried out by qualified staff of the provincial or territorial egg board;
- ≥60 sites sampled per flock; focus on sampling dust and egg conveyances (swabs, dust, fluff, scrapings). Dust samples should be emphasised in the sampling plan as they have been shown to be an important reservoir of *Salmonella*;
- Additional sampling of rodent droppings and dead insects, if found;
- A minimum of 4 composited samples should be tested (that is, the samples from up to 15 separate sites may be composited); and
- Testing must be performed by an accredited laboratory using a cultural method for the isolation of *Salmonella* spp. approved by the Chief Veterinary Officer of each province.

If *S*. Enteritidis is detected in flocks, the eggs from that flock must be diverted for further processing for the lifetime of the flock. If detected in pullet flocks, the pullets should not be

[78] https://www.australianeggs.org.au/for-farmers/tools-and-training/salmonella-risk-assessment-toolkit/post-grading-processes; accessed 14 April 2023
[79] https://inspection.canada.ca/animal-health/terrestrial-animals/hatcheries/canadian-hatchery-and-supply-flock-testing-standar/eng/1583865875096/1583865875440; accessed 13 April 2023

supplied to egg producers for use as laying hens. Following depopulation, the environment should be retested and test negative for *Salmonella* before repopulation. They also recommend that layer flocks that are moving into houses, for which the previous flock tested positive for *S*. Enteritidis, should be vaccinated.

The programme also recommends that egg producers supplying shell eggs for the table market should participate in a HACCP-based On-Farm Food Safety program designed to reduce *S*. Enteritidis, and to address multiple potential sources of *S*. Enteritidis.

In 2015, the joint industry-Canadian Government *Salmonella* Enteritidis Working Group outlined a National Strategy for the Control of Poultry-Related Human *S*. Enteritidis Illness.[80] The aim of this was to reduce the burden of poultry-related human *S*. Enteritidis illnesses caused by both poultry meat and eggs. Five strategic priorities were identified including monitoring and surveillance of *S*. Enteritidis in poultry and poultry products; integrating data from the monitoring and surveillance of *S*. Enteritidis in live poultry, food and human illness; on-farm and industry control strategies; regulatory/policy actions to improve control of *S*. Enteritidis in poultry; and food safety education.

There are also specific requirements for processing of eggs and egg products under Safe Food for Canadians Regulations (SOR/2018-108), gazetted in 2018.[81,82] These include egg washing, conveyance, storage and pasteurisation. Regarding storage, eggs must be held at a licensed egg facility at a maximum temperature of 10˚C or 13˚C depending on the egg grade, and at a maximum relative humidity of a of 85%.

C.2.3 EU

Several EU regulations exist to prevent *Salmonella*-contaminated eggs from being placed on the market.[83] These primarily focus on controlling *Salmonella* in eggs by reducing the prevalence of *Salmonella* amongst layer flocks. Regulation (EC) No. 2160/2003 provided the framework to set EU-wide targets for the reduction of "All *Salmonella* serotypes with public health significance" in laying hens, and for EU Member States to establish national control programmes for *Salmonella*.[84]

Community targets were initially set in Regulation (EC) No. 1168/2006 for the reduction of *Salmonella* Enteritidis and *Salmonella* Typhimurium in adult laying hens of *G. gallus*.[85] The EU target for each Member State was an annual minimum percentage of reduction of positive flocks of adult laying hens by 10 to 40% depending on the prevalence in the preceding year, i.e. Member States were expected to reduce the prevalence each year. Alternatively, Member

[80] https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/national-strategy-control-poultry-related-human-salmonella-enteritidis-illness-canada.html; accessed 13 April 2023
[81] https://laws-lois.justice.gc.ca/eng/regulations/SOR-2018-108/index.html; accessed 13 April 2023
[82] https://inspection.canada.ca/preventive-controls/eggs-and-processes-egg-products/eng/1524259297433/1524259297745; accessed 13 April 2023
[83] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=LEGISSUM:f83005; accessed 13 April 2023
[84] https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:325:0001:0015:EN:PDF; accessed 13 April 2023
[85] https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:211:0004:0008:EN:PDF#:~:text=A%20laying%20flock%20shall%20be,samples%20in%20the%20laying%20flock.; accessed 13 April 2023

States could reduce the maximum percentage to 2% or less. Regulation (EC) No. 1168/2006 was repealed by Regulation (EC) No. 517/2011, but the targets remained the same other than a requirement for Member States to include monophasic *S*. Typhimurium strains with the antigenic formula 1,4,[5],12:i:- within the *S.* Typhimurium total.[86]

The national control programmes may vary to some extent between EU countries but they are based on the same principles and aims (Hugas and Beloeil 2014). The programmes typically include systematic implementation of preventative flock infection measures and surveillance of *Salmonella* within a flock. If *Salmonella* infection is detected, control measures to prevent the spread of infection are implemented. Flocks are tested for the target *Salmonella* serotypes at fixed stages of the production at farms or hatcheries using harmonised sampling plans and standardised analytical methods.

Regulation (EC) No. 1237/2007 sets out specific requirements for the use of eggs that may be contaminated with *Salmonella*.[87] In particular, eggs may only be used for human consumption if they have been treated to destroy all *Salmonella* serotypes with public health significance.

In addition to controls for layer farms, the EU has set targets and controls for breeding flocks of *G. gallus* initially through Regulation (EC) No. 1003/2005, with amendments through Regulation (EC) No. 200/2010.[88,89] The target and controls are for five *Salmonella* serotypes of public health significance: Enteritidis, Hadar, Infantis, Typhimurium and Virchow. The Community target is a reduction of the maximum percentage of adult breeding flocks comprising at least 250 birds remaining positive (for these serotypes) to 1% or less. For Member States with fewer than 100 breeding flocks, not more than one adult breeding flock shall remain positive (for these serotypes) per year.

Table 23 summarises the *Salmonella* sampling scheme for breeding flocks and pullets. The data are collected and reported by EFSA.

[86] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02011R0517-20190310; accessed 13 April 2023
[87] https://www.fsai.ie/uploadedFiles/Legislation/Food_Legisation_Links/Zoonoses/Commission_Regulation_EC_No_1237_2007.pdf; accessed 13 April 2023
[88] https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32005R1003; accessed 13 April 2023
[89] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02010R0200-20190310; accessed 13 April 2023

Table 23. *Salmonella* sampling scheme for breeding flocks and pullets of *G. gallus* for EU Member States.

Target set for:	Target *Salmonella* serotypes	Target	Sampling	Regulation
Hatchery or holding	Enteritidis Infantis Hadar Typhimurium Virchow	Target: Maximum of 1% remaining positive of adult breeding flocks comprising at least 250 birds; for Member States with <100 breeding flocks, not more than one adult breeding flock shall remain positive.	• Frequency: every 2 weeks from hatchery or holding (initiative of operator); every 16 weeks (competent authority). • Hatchery samples: composite soiled hatcher basket liners from 5 baskets or locations, or hatcher swab, or composite broken eggshells from 25 hatcher baskets and up to 5 hatchers. • Breeder facility samples: pooled faeces (number depends on flock size), boot swabs (x5) or boot swab (x1) + dust samples (x1). For cage flocks, 2x150g pooled faeces sample. • Testing: 1 isolate serotyped per *Salmonella*-positive sample. • Considered positive if at least 1 positive sample, or if antimicrobials or bacterial growth inhibitors detected in flock (even if sample negative for *Salmonella*).	200/2010[1]
Adult laying hens	Enteritidis Typhimurium	Target: Maximum 2% remaining positive; for Member States with <50 flocks, not more than one adult flock may remain positive.	• Frequency: o food business operator: every 15 weeks; first sampling at flock age of 24±2 weeks of age; o competent authority: 1 flock per year per holding, at 24±2 weeks of age if *Salmonella* detected in previous flock, in case of foodborne outbreak investigations, in all other layer flocks if *S*. Enteritidis or *S*. Typhimurium detected in one layer flock, or when competent authority considers it appropriate. • Sample type: o Cage flocks: 2x150g pooled faeces or manure belt swabs; o Barn and free range flocks: 2 pairs of boot swabs; may be pooled (operator or competent authority); or 1 pair of boot swabs and 1 dust sample (100g; competent authority).	517/2011[2]

[1] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02010R0200-20190310
[2] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32010R0200&qid=1674004202743

EU regulations regarding egg storage (EC Regulation No. 589/2008)[90] focusses on avoiding the microbiological risk which results from the condensation of atmospheric moisture on the surface of chilled eggs after their removal outside a refrigerated warehouse or retail refrigerator. For that purpose, the regulation stipulates that:

- Eggs should not be refrigerated before sale to the final consumer;
- Class A eggs are to be downgraded to Class B if chilled below 5˚C during a period longer than 24 hours for transport or 72 hours for retail;
- Only eggs destined for French overseas departments be chilled; packages should prominently be marked *'chilled eggs'* (similarly to a dangerous good);
- While a constant temperature of storage and transportation is recommended, there is no specified any concrete value.

[90] https://faolex.fao.org/docs/pdf/eur80074.pdf; accessed 25 May 2023

C.2.4 UK

The UK National Control Programme for *Salmonella* in layer chickens was published in 2007 and came into effect in 2008 (Department for Environment Food and Rural Affairs 2007). Like the EU, the purpose of the programme was to meet a target level set out in Regulation (EC) No 1168/2006 for *S*. Enteritidis and *S*. Typhimurium. The programme covers all layer flocks with ≥350 birds.

Sampling for the programme is aligned with that conducted by the EU. Samples are collected by the operator from day-old chicks to be reared for the production of eggs for human consumption, approximately two weeks before the birds come into lay, or before being moved to laying houses, and then at 15 weeks intervals during the egg laying phase, with the first sample taken when the birds are 22 to 26 weeks of age.

If *S*. Enteritidis or *S*. Typhimurium is detected from environmental samples in a layer flock, the flock is then tested by a Competent Authority. This testing includes two pairs of boot swabs per house (or equivalent faeces samples) plus one dust sample of at least 100 g (or equivalent faeces or boot swabs if dust is not available). If these test positive (and are not the vaccine strain), all eggs from the positive flock headed for human consumption must be heat-treated for the duration of that flock, and advice is provided around strategies to eliminate *Salmonella*. Note if only dust samples test positive but faeces/boot samples test negative, the flock remains under official control, but eggs may continue to be sent direct for human consumption. Official samples of boot swabs (or equivalent faeces) and dust are then taken at two-week intervals for analysis. If the operator/owner of the layer flock disputes the results of the official test they may arrange to have samples taken and tested for *S*. Enteritidis or *S*. Typhimurium of either the caecae and oviducts from 300 birds in the flock selected under supervision of the Competent Authority, or 4000 eggs.

A majority (>90%) of eggs produced in the UK are also under the British Lion scheme, which was launched in 1998.[91] The Code of Practice for the scheme covers the entire production chain and incorporates food safety controls above and beyond those outlined in current UK and EU legislation (The British Egg Industry Council 2013). Some features of the scheme Code of Practice include:

- Vaccination of hens against *S.* Enteritidis and *S.* Typhimurium (under the UK National Control Programme, this is common-place, but not mandatory);
- Registration and a unique 'passport' system, ensuring complete traceability of hens, eggs and feed;
- Increased hygiene controls and *Salmonella* testing of all flocks in the integrated egg production chain, in excess of the National Control Programme, including turnaround swabbing of breeding, pullet rearing and layer flocks; and packing centre hygiene swabbing;
- Regular egg testing (includes testing of at least 20 eggs per quarter from each Lion farm);
- Stringent feed controls;
- Lion Quality eggs stamped on farm with the farm code and production method;
- Storage at below 20˚C, with storage instructions for the consumer or caterer to “keep refrigerated after purchase”;

[91] https://www.egginfo.co.uk/british-lion-eggs; accessed 13 April 2023

- Best-before date and Lion logo printed on the shell of Lion Quality eggs as well as on the egg box; and
- Regular independent auditing, including unannounced audits, of all producers and packers in the Lion scheme, in accordance with the ISO 17065 standard.

C.2.5 US

In the US, approximately 70% of eggs are sold as shell eggs (both pasteurised and non-pasteurised), while the remainder are processed into liquid, frozen or dried, pasteurised egg products. The majority of egg products are destined for institutional use or further processing into foods such as cake mixes, pasta, ice cream, mayonnaise, and bakery goods. The pasteurisation process is designed to achieve a 5-log reduction of any *S*. Enteritidis that may be present in eggs.

The US FDA Egg Safety Final Rule was gazetted on 9 July 2009 and was effective from 10 July 2010.[92] No changes to this Rule were identified since that time, although some flexibility to requirements was allowed over the duration of the US public health emergency as a result of COVID-19.[93] The Rule requires producers with >3000 layer hens to register a *S*. Enteritidis prevention plan for production, storage, and transport of shell eggs. It is primarily focused on preventing transovarian transmission, and growth within the egg during storage.

The Rule requires that egg producers whose shell eggs are not processed with a treatment such as pasteurisation must:[94]

- Procure chicks and pullets from suppliers who monitor for *S*. Enteritidis.
- Establish rodent, pest control, and biosecurity measures to prevent spread of *S*. Enteritidis throughout the farm by people and equipment.
- Conduct environmental testing for *S.* Enteritidis. Samples typically consist of gauze swabs dragged over manure, with each sample covering a row/bank of the shed. Sampling is conducted:
  - When pullets are 14-16 weeks of age.
    - If negative, the environment of the flock is tested again at 40-45 weeks.
    - If positive, cleaning and disinfection of pullet environment required.
  - Four-to-five weeks after induced moult.
  - If the environment tests positive at any stage, eggs must be diverted for treatment for the life of the flock. Alternatively, eggs can be tested (within two weeks of start of lay if pullets test positive within 10 days of positive environmental test result). Testing includes four egg tests (1,000 eggs/test) at two-week intervals (4,000 eggs total).
    - If all negative, no more egg testing required.
    - If any are *S*. Enteritidis-positive, all eggs must be diverted for additional treatment until four egg tests at two-weekly intervals test negative. If this occurs, eggs may be sold untreated but must be tested monthly (1,000 eggs/test).
- Clean and disinfect poultry houses that have tested positive for *S*. Enteritidis.

[92] https://www.govinfo.gov/content/pkg/FR-2009-07-09/pdf/E9-16119.pdf; accessed 11 April 2023
[93] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/temporary-policy-regarding-enforcement-21-cfr-part-118-egg-safety-rule-during-covid-19-public-health; accessed 12 April 2023
[94] https://wayback.archive-it.org/7993/20161022183901/http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM232271.pdf; accessed 11 April 2023

- Refrigerate eggs at 45 ˚F (7 ˚C) during storage and transportation no later than 36 hours after the eggs are laid (this requirement also applies to egg producers whose eggs receive a treatment, such as pasteurisation).
- Required written records include:
  - *S.* Enteritidis prevention plan and records documenting their compliance.
  - Documentation that pullets were raised under "*S*. Enteritidis-monitored" conditions;
  - Environmental and egg sampling procedures;
  - Results of *S*. Enteritidis testing;
  - Diversion of eggs;
  - Eggs at a particular farm being given a treatment; and
  - Records of review and of modifications of the *S*. Enteritidis prevention plan and corrective actions taken.
  - Records documenting compliance with the *S*. Enteritidis prevention measures, as follows:
    - Biosecurity measures.
    - Rodent and other pest control measures.
    - Cleaning and disinfection procedures performed at depopulation.
    - Refrigeration requirements.