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

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DISCLAIMER

ABBREVIATIONS

1997NNS	The 1997 National Nutrition Survey
2002CNS	The 2002 National Childrens' Nutrition Survey
2009ANS	The 2009 Adult Nutrition Survey
CFU	Colony forming unit
CI	Confidence interval
DNA	Deoxyribose nucleic acid
EFSA	European Food Safety Authority
EU	European Union
FSANZ	Food Standards Australia New Zealand
MAF	Ministry of Agriculture and Forestry (New Zealand)*
MLVA	Multiple-locus variable-number tandem repeat analysis
MLST	Multilocus sequencing typing
MPI	Ministry for Primary Industries (New Zealand)*
MPN	Most probable number
NZFSA	New Zealand Food Safety Authority*
OR	Odds ratio
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
pH	Measure of acidity (min. = 0 = most acidic; max. = 14)
RMQ	Risk management question
SNP	Single nucleotide polymorphism
UK	United Kingdom
USFDA	United States Food and Drug Administration
VNTR	Variable number tandem repeats
WGS	Whole genome sequencing
YMT	Yolk membrane time

* On 1 July 2010, NZFSA and MAF were amalgamated. On 30 April 2012, MAF was renamed as MPI. This document uses the names NZFSA and MAF for documents produced during the existence of these organisations.

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SUMMARY

This Risk Profile considers nontyphoidal *Salmonella enterica* subspecies *enterica* (hereafter referrred to as *Salmonella*) in or on eggs. This is an update of a Risk Profile published in 2011.

Salmonella can colonise poultry and can be released into the environment with poultry manure. Eggs can become contaminated with *Salmonella* prior to laying (i.e. during formation and passage inside the bird) or after laying (i.e. contamination from the environment).

Salmonella of the 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 is not considered to be endemic in New Zealand and is currently not considered a public health concern in this country.

The serotype *S*. Typhimurium is more common amongst human infections in New Zealand. This serotype seldom colonises the reproductive organs of laying hens nor contaminates the eggs prior to shell formation (although it is able to do these things), and typically contaminates the surface of eggs or penetrates through the formed shell into the contents.

Whole eggs inhibit bacterial contamination of the contents through physical barriers (cuticle, shell, membranes) and antimicrobial components in the albumen. The egg yolk supports *Salmonella* growth. *Salmonella* may reach the egg yolk by migrating through the egg and across the vitelline membrane surrounding the yolk, encountering the yolk as the vitelline membrane breaks down over time, or when eggs are broken and their contents released.

The New Zealand layer industry is large, producing approximately one billion eggs per year from chickens. National egg production from flocks of poultry in New Zealand other than chickens is not known. The majority of eggs are sold as fresh, whole eggs in New Zealand but liquid and dried egg products are also available.

The purpose of the Risk Profile is to critically review new information to answer the following Risk Management Questions (RMQs):

1. Has the public health risk from *Salmonella* in or on eggs consumed in New Zealand changed since the 2011 Risk Profile?

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

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

4. What is the best way to gather information on the prevalence of *Salmonella* in New Zealand eggs?

RMQ1: Has the public health risk from Salmonella in or on eggs consumed in New Zealand changed since the 2011 Risk Profile?

From available data, the public health risk from *Salmonella* in or on eggs consumed in New Zealand has not changed since the 2011 Risk Profile, i.e. there is little evidence that transmission of *Salmonella* via eggs is a significant transmission route occurring in New Zealand.

However, there is evidence to show that whole, fresh eggs sold in New Zealand can be contaminated with *Salmonella* and this may be contributing to a small (but undefined) proportion of human illness:

- Eggs produced in New Zealand can potentially be externally contaminated by *Salmonella*. *Salmonella* have been isolated from the shells of whole, fresh eggs purchased at retail in New Zealand (1.8% in the most recent 2007 survey). Experimental results show that salmonellae could survive for a month or more on the shell of whole eggs, indicating capacity to survive from point-of-lay to point-of-consumption. There are no data on *Salmonella* prevalence in New Zealand layer flocks or layer farm environments.
- *Salmonella* have not been isolated from egg contents in any New Zealand surveys and *S.* Enteritidis has not been isolated from eggs in any New Zealand surveys. Experimental evidence shows that internal contamination is possible for some non-Enteritidis serotypes but migration through the shell, survival and growth are subject to storage temperature (all are accelerated with increased temperature).
- Time/temperature data for whole, fresh eggs from the point-of-lay to the point-of-consumption are not available for New Zealand. Growth of *Salmonella* in the contents of eggs appears to be supported at temperatures of 7°C or above (data between 4 and 7°C are needed). Whole, fresh eggs may be kept at these temperatures at any point along the food chain but the combination of temperature and time is important for assessing the potential for growth.
- There have been salmonellosis outbreaks reported in New Zealand where there was strong evidence to implicate eggs as the vehicle of infection (6 outbreaks in 15 years).

Data from national nutrition surveys indicate that eggs are consumed by almost half of New Zealanders each day. The risk of illness if *Salmonella* are present will be mitigated because the majority of egg servings are cooked and only a very small proportion of servings appear to be consumed raw. Some egg cooking processes will be insufficient to eliminate any *Salmonella* present. *Salmonella* contaminating an egg shell could cause illness if introduced to other foods (pooled eggs or cross-contamination) or may pose a risk for the food handler (e.g. touching mouth after shelling eggs). Kitchen surfaces and utensils may become contaminated by raw contaminated eggs and cross-contaminate other foods.

There is not enough data to assess the risk from liquid or dried eggs. It appears that egg pasteurisation regimes recommended for use in New Zealand would inactivate any *Salmonella* present in egg contents, but further validation would provide better assurance.

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

There are multiple interventions that can be applied on-farm, but prevention and control of *Salmonella* is best achieved through a comprehensive programme incorporating multiple controls. Vaccination is recommended in New Zealand but not compulsory. Feeding prebiotics and probiotics to hens has been shown to provide some protection against *Salmonella*. Environmental management includes controlling the food and water supply, biosecurity and pest management, and ensuring effective cleaning regimes are in place.

Maintaining refrigeration of eggs post-lay will control the growth of any *Salmonella* that might be present in the egg contents. Egg washing/sanitising is optional in New Zealand, but international opinion differs with respect to the effectiveness of this intervention in controlling *Salmonella*.

The information available on interventions is extensive and needs to be assessed for applicability in the New Zealand context. A separate, more comprehensive review of the efficacy of intervention options relevant to New Zealand is recommended.

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

In addition to the information collated in this Risk Profile, the Ministry for Primary Industries (MPI) has published a review that examined whether the number of salmonellosis cases attributed to eggs would increase if the shelf life of eggs were extended to 35 days, irrespective of temperature. MPI concluded that "it would appear prudent to maintain the current requirements for handling and storage of eggs". The New Zealand Risk Management Programme for eggs sets out temperature controls for eggs, which requires temperatures to be maintained at 15°C or below for eggs stored up to 35 days post-lay.

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

Environmental sampling at layer farms more efficiently and effectively detects the potential for *Salmonella* to contaminate eggs. An effective sampling regime will include both faeces and dust, and will maximise the number of samples taken. A separate study is recommended to understand the relationship (if any) between the results of environmental surveys of layer housing and the prevalence of *Salmonella* on eggs in New Zealand. Such a study could investigate the relationship between a *Salmonella*-positive flock and *Salmonella*-positive eggs. Mathematical modelling to predict the likely prevalence of *Salmonella*-positive eggs in New Zealand, given a prevalence of *Salmonella*-positive flocks would also inform shelf life considerations.

1. INTRODUCTION

This document updates the 2011 Risk Profile considering *Salmonella* (non typhoidal) in and on eggs from chickens and other poultry such as ostriches, ducks, and quail (Lake *et al.*, 2011). This Risk Profile only considers bacteria classified as *Salmonella enterica* subspecies *enterica*, excluding the typhoidal serotypes Typhi and Paratyphi. For simplicity, the term "*Salmonella*" is used throughout this document to only refer to this *Salmonella* subspecies (unless otherwise stated).

This is not a stand-alone document and readers are referred to the 2011 Risk Profile, which can be accessed from: http://www.foodsafety.govt.nz/science-risk/risk-assessment/risk-profiles/.[1]

The purpose of this update is to critically review new information to answer the following risk management questions:

1. Has the public health risk from *Salmonella* in or on eggs consumed in New Zealand changed since the 2011 Risk Profile?
2. What interventions are available to manage the risk from *Salmonella* in and on eggs and what is known about their effectiveness?
3. What information is available to advise industry regarding shelf life and storage conditions for eggs in relation to the risk from *Salmonella*?
4. What is the best way to gather information on the prevalence of *Salmonella* in New Zealand eggs?

In 2015, the Ministry for Primary Industries (MPI) finalised a review on the horizontal transfer and growth of *Salmonella* in eggs in New Zealand (Ministry for Primary Industries (MPI), 2015). This review was prepared based on information available up until 2011. This Risk Profile update therefore includes relevant information since 2011 that is within the scope of the MPI review, as well as other information published between 2011 and 2015.

Risk Profiles provide scientific information relevant to a food/hazard combination for risk managers and describe potential risk management options.[2]

[1] Accessed 10 December 2015.
[2] http://www.foodsafety.govt.nz/elibrary/industry/RMF_full_document_-_11604_NZFSA_Risk_Management_Framework_3.1.pdf (accessed 10 December 2015).

2. HAZARD AND FOOD

2.1 THE PATHOGEN: *SALMONELLA*

Appendix A.1 contains information on Salmonella typing methods.

Key findings

All *Salmonella* serotypes are considered potentially pathogenic to humans, except for the few that are specific to certain animal hosts. Pathogenicity varies between and within serotypes, but is not yet predictable.

Salmonella enterica subspecies *enterica* 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 is not considered to be endemic in New Zealand and is currently not considered a public health concern in this country.

The serotype *S*. Typhimurium is more common amongst human infections in New Zealand. This serotype seldom colonises the reproductive organs of laying hens nor contaminates the eggs prior to shell formation (although it is able to do these things), and typically contaminates the surface of eggs or penetrates through the formed shell into the contents.

General information on the growth, survival and inactivation of *Salmonella* is presented in the 2011 Risk Profile and microbiological datasheets available from

http://www.foodsafety.govt.nz/science-risk/hazard-data-sheets/pathogen-data-sheets.htm[3]

and a more recent document published by Food Standards Australia New Zealand (FSANZ), available from:

http://www.foodstandards.govt.nz/publications/pages/agentsoffoodborneill5155.aspx[4]

The nomenclature of *Salmonella* spp. has not changed since the 2011 Risk Profile. *Salmonella* spp. serotypes relevant to food safety most often belong to the *Salmonella enterica* subspecies *enterica* group, which includes more than 2,400 serotypes (Brenner *et al.*, 2000).[5] *Salmonella* serotypes are commonly shortened to include the non-italicised serotype name, e.g., *Salmonella enterica* sub species *enterica* Enteritidis is referred to as *Salmonella* Enteritidis or *S.* Enteritidis (Grimont and Weill, 2007).

All *Salmonella* serotypes are considered potentially pathogenic to humans, except for those specific to certain animal hosts (e.g. the poultry-specific *Salmonella* Gallinarum-Pullorum) (EFSA Panel on Biological Hazards (BIOHAZ), 2010).[6] It appears that pathogenicity can vary between serotypes although this is not yet predictable. An Australian study showed that the

[3] Accessed 16 November 2015.
[4] Accessed 7 October 2015.
[5] The terms "serotype" and "serovar" are interchangeable. "Serotype" is used in this document.
[6] *S.* Gallinarum-Pullorum was previously separated into *S.* Gallinarum and *S.* Pullorum. *S.* Gallinarum has not be reported in New Zealand and *S.* Pullorum was last reported in 1985 (MAF biosecurity, 2009).

pathogenicity of four strains of *Salmonella* Typhimurium and six other (non-Enteritidis) serotypes (all isolated from layer farms) was variable, but overall the Typhimurium serotypes exhibited the greatest invasion of human intestinal cell lines and were the only serotypes to cause disease in mice (McWhorter and Chousalkar, 2015). These findings were confirmed in a second study of 17 different *Salmonella* serotypes by the same group (McWhorter *et al.*, 2015). Molecular analysis of five pathogenicity islands could not identify specific genomic changes that could be related to pathogenicity, and the authors suggested that multiple changes were responsible for the observed responses, although they cautioned that within-serotype differences in pathogenicity are also likely (McWhorter *et al.*, 2015).

The primary sources of *Salmonella* are the gastrointestinal tracts of humans and animals and the widespread presence of the organism in the environment is due to direct or indirect faecal contamination (Bell and Kyriakides, 2002). Salmonellae may be transmitted to humans via person-to-person transmission, contaminated food or water, animal contact or from a contaminated environment (Silva *et al.*, 2014). A review of non-typhoidal salmonellosis sporadic cases and outbreaks in New Zealand from 2000 to 2009 indicated that the most important pathway for *Salmonella* transmission was consumption of food, and although there were insufficient data to identify the most important foods, infected food handlers were identified in approximately half the outbreaks (Adlam *et al.*, 2010). Other pathways were consumption of untreated drinking water and contact with animals, while person-to-person transmission and overseas travel were less important for New Zealand.

Salmonellae remain a serious cause of foodborne illness worldwide. In the European Union (EU) and in North America, serotypes Enteritidis and Typhimurium are reported as the two major aetiologic agents of salmonellosis that have adapted to humans (Centers for Disease and Prevention, 2013; Crim *et al.*, 2014; ECDC, 2015; Taylor *et al.*, 2012).

S. Enteritidis can colonise the ovaries of chickens and contaminate eggs prior to shell formation and this serotype causes the majority of salmonellosis cases attributed to eggs in Europe and North America (EFSA Panel on Biological Hazards (BIOHAZ), 2014; Martelli and Davies, 2012). It is also the serotype most commonly found in laying flocks in Europe and North America (EFSA Panel on Biological Hazards (BIOHAZ), 2010). Consequently, international research into *Salmonella* in and on eggs has largely focussed on *S.* Enteritidis. However, in New Zealand, *S.* Enteritidis has not been identified as endemic, and it is also infrequently detected in Australia and is therefore not currently considered to be a public health concern in Australia or New Zealand (Ministry for Primary Industries (MPI), 2015).

S. Typhimurium is also associated with eggs, and is the serotype most commonly found in laying hens and eggs in non-European countries (Chousalkar and Roberts, 2012; EFSA Panel on Biological Hazards (BIOHAZ), 2014; Jamshidi *et al.*, 2010). *S.* Typhimurium is more important in New Zealand in terms of human illness (see Section 3.3.4). Unlike *S.* Enteritidis, *S.* Typhimurium very seldom colonises the ova or oviduct of laying hens, although it is able to do this. Egg contamination by *S.* Typhimurium occurs most often by egg shell penetration and/or surface contamination by faecal matter, either in the laying environment or whilst processing eggs (EFSA Panel on Biological Hazards (BIOHAZ), 2010). Other non-Enteritidis *Salmonella* serotypes (e.g. Senftenberg, Livingstone, Infantis) have been occasionally isolated from eggs, mainly from egg shells and rarely from egg contents (Chousalkar and Roberts, 2012; Gole *et al.*, 2014a; Martelli and Davies, 2012; Shirota *et al.*, 2012). Atypical pathogenic *Salmonella* (i.e. non-motile variants of *S*. Typhimurium) have also been reported from human salmonellosis cases and found in laying hens in Europe (France) (Le Hello *et al.*, 2012).

Since the 2011 Risk Profile, research on *Salmonella* continues to be dominated by studies of *S*. Enteritidis contamination in eggs and infections in humans although there is a growing amount of research that is including *S*. Typhimurium. A review cited in the 2011 Risk Profile concluded that (Wales and Davies, 2011):

- Based on *in vivo* challenge studies, some strains of *S*. Typhimurium appear to have similar capabilities to *S*. Enteritidis in respect of intestinal colonisation and systemic infection of laying hens, survival in the forming and laid egg, and penetration of eggshells and membranes; however
- It appears that *S.* Enteritidis is better able to avoid the host immune response and persistently colonise the ovary and oviduct of hens compared to *S.* Typhimurium, and *S.* Enteritidis is also more likely to be detected in egg contents.

Publications since 2011 are consistent with these findings. Therefore, this Risk Profile uses information generated through studies of both *S.* Enteritidis and *S.* Typhimurium, where information from the former serotype helps to inform worse-case scenarios for the latter serotype.

2.2 THE FOOD: EGGS

Key findings

The egg yolk supports bacterial growth. Whole eggs inhibit bacterial contamination of the contents through physical barriers (cuticle, shell, membranes) and antimicrobial components in the albumen.

Available data suggests that the size of the chicken egg producing industry has not changed since the 2011 Risk Profile. The industry is large, producing approximately one billion eggs per year. There are no collated data on egg production from flocks of poultry in New Zealand other than chickens. A new Code of Welfare requires changes to conventional egg production facilities and this will impact egg production in coming years.

The majority of eggs are sold as fresh, whole eggs in New Zealand but liquid and dried egg products are also available. The amount of egg products exported from New Zealand has increased since 2010, but the export market is modest (approximately 2,000 tonnes in recent years, mostly whole, fresh eggs). Approximately 250 tonnes were imported in recent years, comprised mostly of dried egg. Fresh eggs were not imported.

Eggs are a popular food not only for their nutritional aspects, but also for their functional properties, e.g. the coagulant capacity of proteins, the foaming capacity of albumen proteins and the emulsifying capacity of the yolk (EFSA and ECDC, 2015). These properties are used in different ways to produce and enrich many types of foods, e.g. pastries, sauces, dressings, desserts and pasta. Eggs are often used raw or only lightly heat-treated.

As reported in the 2011 Risk Profile, the majority of eggs are marketed and consumed as fresh shell eggs but liquid eggs and dried egg are also available in New Zealand (see Section 2.2.1). The egg yolk is a nutritious medium for bacterial growth.

A useful description of the physiology of egg formation and laying has been recently published by Howard *et al*. (2012). Eggs are passed by hens through the same opening as is used to eliminate faecal matter (the vent), but a bird cannot perform both functions at the same time (Howard *et al.*, 2012).

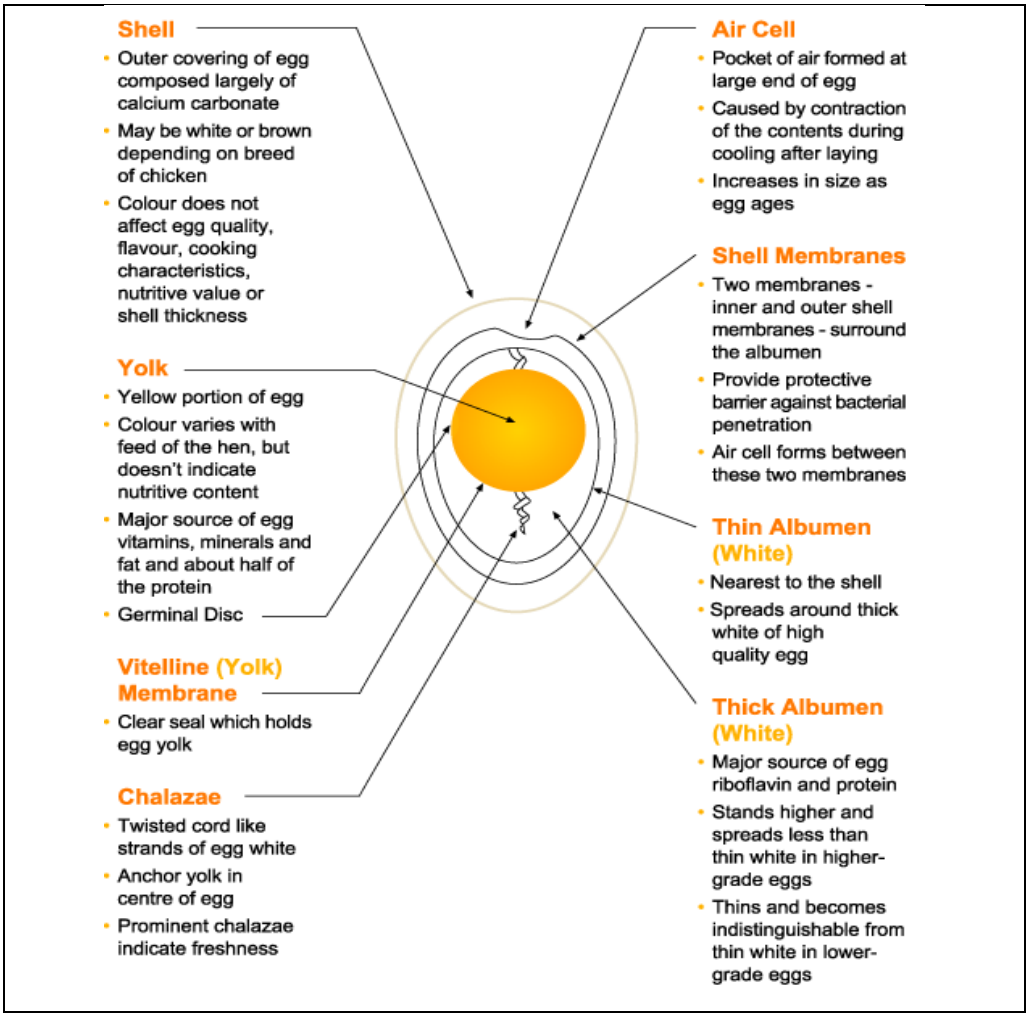

FIGURE 1: Egg anatomy*

*Image reproduced with permission from Eggs Incorporated (New Zealand).[7] Image available at http://www.eggs.org.nz/whats-in-an-egg/ (accessed 16 November 2015).

FIGURE 1 shows the major components of an egg. The cuticle, shell and associated membranes create physical barriers to inhibit bacterial contamination of the egg contents and antimicrobial components are present in the egg white (albumen) (Howard *et al.*, 2012):

- 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) into the egg. Two membranes under the shell together provide the third outer barrier. The vitelline membrane surrounds the yolk and acts as the final barrier between invading bacteria and the nutrient-rich yolk.

[7] Eggs Incorporated (NZ) manages promotional activities for the New Zealand Egg Producers Federation (http://eggfarmers.org.nz/about-eggs/about-epf, accessed 16 November 2015).

- Antimicrobial components: The iron-chelator ovotransferrin, the proteinase inhibitor ovomucoid, the biotin binder avidin and the enzyme lysozyme. 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 inibitory to *Salmonella* growth (Silversides and Budgell, 2004).

It is difficult for bacteria to move across an intact good quality egg shell but small defects in the shell increase the opportunity for bacteria to penetrate and move into the egg contents (Samiullah *et al.*, 2013).

2.2.1 Egg production in New Zealand

Information on the chicken layer industry in New Zealand is available through the website of the Egg Producers Federation of New Zealand and additional information was kindly provided by this organisation.[8] Comparison with data reported in the 2011 Risk Profile (from the same website) suggests little change to the size of the industry in the last five years:

- The number of commercial egg producers is slightly lower (143);
- The majority of eggs produced in New Zealand are still from conventional cage production systems, although this proportion has decreased (as at 30 June 2015, 78% of the flock was conventionally caged, 17.5% free-range, 3% barn and 1.5% colony caged);
- The number of layer hens is still around three million (as at 30 June 2015, the national flock of layer hens was estimated at 3.48 million birds)
- The number of eggs produced each year remains the same (approximately 1 billion);
- The majority of eggs (85%) are still sold as table eggs.

Approximately 18 farms produce 85% of the eggs produced in New Zealand.

The majority of commercial layer chickens in New Zealand are either Hyline Brown or Brown Shaver varieties and these hens typically lay 320 eggs per year. They can begin laying at 18 weeks and are capable of laying up until 80 weeks of age.[9] The laying cycle is typically 67 weeks before hens naturally moult, at which time the hens are culled.

Relatively minor amounts of chicken eggs are also produced from small flocks kept by people in towns or on farms. Some of these eggs may be sold to the public, e.g. through farm stores or the internet.

No consolidated data are available on egg producers farming other types of poultry in New Zealand.

A new Code of Welfare for layer hens was introduced in 2012 (National Animal Welfare Advisory Committee, 2012). Under this code the conventional cages used for layer hens will gradually be replaced (and cannot be used after 2022) with colony cages, free-range or barn systems. Colony cages are also referred to as enriched or furnished cages, and have perching, scratching and nesting areas. The shift from conventional cages to the other production systems increases the space required per bird so existing facilities will hold fewer birds in the same area. The response of egg producers will determine how the new Code will

[8] http://eggfarmers.org.nz/ (accessed 16 November 2015). The EPFNZ is the trade association representing commercial egg farmers, i.e. farmers who own 100 or more layer hens and sell eggs.
[9] A chick is a baby bird aged up to seven weeks (day-old chicks are chicks up to 72 hours of age that are surviving on their internal yolk sack), and a pullet is a layer aged between seven weeks and the time when the hen begins to lay eggs (National Animal Welfare Advisory Committee, 2012).

affect egg production in New Zealand, but a decrease in egg supply (and increase in egg prices) has been predicted.[10]

Liquid egg (fresh or pasteurised, whole or separated, with or without other ingredients) and dried egg are manufactured in New Zealand but the amount sold through New Zealand wholesale or retail outlets is not known. Some product is sold frozen. The products are most often used by commercial food manufacturers or food service businesses but are also available to the public.

2.2.2 International trade

The amount, by weight, of whole eggs and egg products exported from New Zealand has increased since 2010 (FIGURE 2).[11] Almost all (99%, by weight) of the whole eggs exported in 2014 and 2015 were fresh (and almost all were eggs from chickens), and almost all (>98%, by weight) of the egg contents were exported as yolks or albumen that had been "cooked by steaming or boiling in water, moulded, frozen or otherwise preserved".[12] Countries encircling and within the Pacific Ocean were the main destinations. For the year ending June 2015, the main export destinations for fresh New Zealand table eggs were New Caledonia (497 tonnes), Papua New Guinea (332 tonnes), French Polynesia (302 tonnes) and Hong Kong (270 tonnes).

The amount of eggs and egg products imported into New Zealand is small relative to exports, but appears to be increasing (FIGURE 2). Whole eggs only make up a small proportion of the total weight of imported egg products (11% for the year ending June 2015) and all of these are preserved or cooked. For the year ending June 2015, the majority (94%) of whole eggs came from the People's Republic of China. The largest proportion of imported egg contents were dried yolks or dried albumen (87% by weight, year ending June 2015), and the remainder comprised of liquid yolks or albumen, or albumen that had been cooked, frozen or preserved. For the year ending June 2015, the main countries of origin for dried egg contents were The Netherlands (90 tonnes), Italy (59 tonnes), Denmark (31 tonnes) and the USA (26 tonnes). Liquid albumen was imported only from Thailand (10 tonnes), and albumen that had been cooked, frozen or preserved was imported from the People's Republic of China (10 tonnes), Thailand (10 tonnes) and Taiwan (2 tonnes).

[10] http://eggfarmers.org.nz/eggfarmers/wp-content/uploads/2012/07/code_economic_impact_summery.pdf (accessed 15 March 2016).
[11] Export and import data cited in this section are from Statistics New Zealand Infoshare (http://www.stats.govt.nz/infoshare/, accessed 16 November 2015).
[12] Assuming a weight of 30g per egg, approximately 56 million fresh chicken eggs were exported during the year ending June 2015. This is 6% of the estimated 1 billion chicken eggs produced in New Zealand per year.

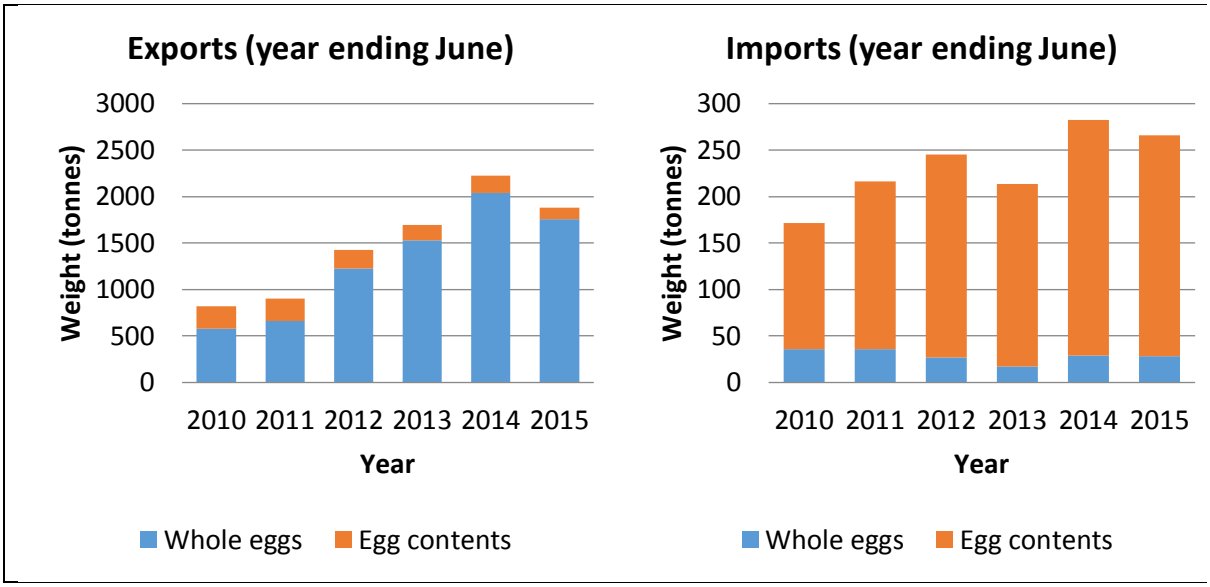

FIGURE 2: Weight of whole eggs and egg products exported into and imported from New Zealand per year*

*Data from Statistics New Zealand Infoshare (http://www.stats.govt.nz/infoshare/, accessed 16 November 2015).

2.2.3 Amount available to the New Zealand consumer

Approximately one billion chicken eggs are commercially produced per year in New Zealand. The available data suggests that approximately 6% of fresh chicken eggs are exported but none are imported. An unknown proportion of fresh chicken eggs are also diverted for making egg products, most of which are pasteurised or dried. This Risk Profile assumes that the majority of chicken eggs produced in New Zealand are sold to domestic consumers as whole, fresh eggs, but the actual amount available is not known.

There are not enough data on eggs from other types of poultry to estimate the amounts available to the New Zealand consumer.

2.3 CONTAMINATION OF EGGS BY *SALMONELLA*

Key findings

Salmonella can contaminate the surface of eggs or the contents. Internal contamination arises through transmission from the infected reproductive organs of a bird before the shell is formed (vertical transmission) or penetration of *Salmonella* through the shell and external membranes (horizontal transmission).

Non-Enteritidis serotypes can colonise the reproductive tissues of laying hens so there is potential for these serotypes to be transmitted vertically. Horizontal transmission has been demonstrated with a wider variety of serotypes.

It has been suggested that poor shell quality increases the opportunity for *Salmonella* to penetrate eggs but the scientific literature does not provide consistent evidence for this.

Salmonella can persist in flocks over time. Investigations into the effects of different flock housing (conventional and enriched cages, barn, free-range) on the prevalence of *Salmonella* in layers and eggs continue to yield conflicting and inconsistent results.

Feed can potentially introduce *Salmonella* into the flock. Available New Zealand data do not suggest animal feed is a major source of human salmonellosis in New Zealand, but comprehensive evidence is lacking.

2.3.1 External and internal egg contamination

As explained in the 2011 Risk Profile, *Salmonella* can contaminate the surface of eggs (external contamination) or the egg contents (internal contamination). An egg contaminated with *Salmonella* will not show any noticeable changes. External faecal contamination will not necessarily be visible.

External contamination of the shell of eggs may arise from infection of the lower reproductive tract of the hen or faecal contamination from hens with gastrointestinal infection with *Salmonella*. Further shell contamination may occur from the environment into which the eggs are laid. External contamination of egg shells 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.

The 2011 Risk Profile described the two possible routes for *Salmonella* to contaminate the egg contents. These were, briefly:

- Trans-ovarian (vertical transmission): Where *Salmonella* colonise the reproductive organs of hens and contaminate the yolk, albumen, egg shell membranes or shell before the egg is layed; and
- Trans-shell (horizontal transmission): Where *Salmonella* penetrate the egg shell and reach the internal contents. The *Salmonella* might originate from the colonised gut of the hen or from faeces that come into contact with the egg during or after laying.

In terms of vertical transmission, *S*. Enteritidis is still considered to be the most common serotype to invade the reproductive tissues of laying hens (EFSA Panel on Biological Hazards (BIOHAZ), 2014), but other *Salmonella* serotypes can infect reproductive tissues, including *S*. Typhimurium and *S*. Heidelberg (Martelli and Davies, 2012; Wales and Davies, 2011).

A wider variety of *Salmonella* serotypes can be involved in the horizontal transmission route (Chousalkar and Roberts, 2012; EFSA Panel on Biological Hazards (BIOHAZ), 2014; Jamshidi *et al.*, 2010; Zhang *et al.*, 2011). Environmental hygiene is critical for controlling *Salmonella* dissemination for this transmission route because the pathogen can survive in the laying house over subsequent flock cycles, therefore posing a contamination risk onto the surface of eggs and potentially penetrating through the eggshell and into the egg contents (Carrique-Mas *et al.*, 2008; Carrique-Mas *et al.*, 2009).

The ability of *Salmonella* on the exterior of eggs to penetrate into the yolk where growth can occur depends on a number of intrinsic factors related to physical barriers and chemical components with antibacterial properties, as described previously (Section 2.2) and in a more recent review (Zhang *et al.*, 2011). The integrity of the cuticle, shell and shell membranes is the most important defence mechanism (Ministry for Primary Industries (MPI), 2015).

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).[13] However, older studies have suggested no relationship between shell quality and internal contamination of *Salmonella*, and some recent studies continue to support this (Section 2.4.2; (Rathgeber *et al.*, 2013)). Older flocks are a risk factor for *Salmonella*

[13] Examples of shell quality measurements include the amount of cuticle present, shell thickness, translucency and breaking strength. Internal egg quality measurements include albumen height, Haugh units and yolk colour.

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 machines which are used to detect cracks in eggs. An EU project has shown that the presence of microcracks in the shell and the absence of the cuticle increase the probability of trans-shell penetration (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, e.g. washing or wiping) can affect the integrity of the intrinsic factors that protect the egg from penetration and growth of bacteria within the egg contents (Ministry for Primary Industries (MPI), 2015). Egg washing is discussed in sections 2.4.2 and 5.2.2.

2.3.2 Contamination at the layer farm

A recent systematic review of studies mostly from the EU and USA identified the following risk factors for *Salmonella* contamination of shell eggs (Denagamage *et al.*, 2015):

- High level of manure contamination with *S.* Enteritidis;
- Middle phase of production (hen age of 35-56 weeks);
- High degree of egg-handling equipment contamination;
- Flock size of >30,000; and
- Egg production rate of >96% (percentage of birds in a flock actively laying eggs).

A larger flock size (>30,000 hens) was also identified as a risk factor for *Salmonella* contamination of laying hen premises, along with:

- The presence of previous *Salmonella* infection;
- Absence of cleaning and disinfection;
- Presence of rodents;
- Induced moulting;
- Multiage 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 farms and air inlets.

Most of these risk factors are not surprising, e.g. poor disinfection and access by pests support persistent *Salmonella* populations.

The persistence of *Salmonella* among flocks is influenced by faecal shedding from infected birds. Faecal shedding of salmonellae is a product of their ability to adhere to cells of the avian intestinal tract (Gast *et al.*, 2015). Intestinal colonisation of *Salmonella* usually declines steadily following experimental infection of mature chickens but can persist for several months.

A longitudinal study using dust, faecal and cloacal samples to monitor *Salmonella* among 41 flocks from different production systems in three European countries found that 10/41 flocks

tested positive at least once during the laying period (up to 60 weeks) (Schulz *et al.*, 2011). A *Salmonella*-positive finding significantly increased the probability of subsequent positive results in the same flock. Generally the same serotypes and phage types were detected within a flock over the laying period. There was no significant difference in prevalence related to age of the flock (other studies have found that older flocks were more likely to be *Salmonella*-positive). The authors noted that farm management practices influenced findings strongly, e.g. *Salmonella* was less likely to be detected on farms where the cages are cleaned and faeces are removed.

Studies in Belgium showed that one or two strains of *Salmonella* can persist on farms through successive layer cycles, despite cleaning and disinfection procedures (Dewaele *et al.*, 2012a; Dewaele *et al.*, 2012b). Multiple sources and transmission routes were identified but the authors suggested that the main reservoirs were the egg collecting areas, henhouses and rodents, and also demonstrated the potential for cross-contamination between farms visited by the same egg buyers. Rodents were associated with *Salmonella* spp. contamination of commercial layer farms in Japan (Lapuz *et al.*, 2012).

A paper cited in the 2011 Risk Profile, which reported that the natural exposure of hens to *S.* Enteritidis via inoculated "seeder" pen-mates was sufficient to generate *Salmonella*-positive eggs, but similar studies for *S.* Typhimurium were lacking (Wales and Davies, 2011). New studies have also been published investigating the spread of *Salmonella* among birds in a flock and its persistence on the layer farm. When four birds of a flock of 200 were inoculated with *S.* Enteritidis at the onset of lay, in general, the inoculum was observed to spread to other birds within the flock and the proportion of positive eggs increased over time (Thomas *et al.*, 2011). A marker strain of *S.* Typhimurium also spread from inoculated birds to non-inoculated birds when these comingled in different housing arrangements (Hannah *et al.*, 2011). Studies of this type on non-Enteritidis serotypes are still rare and may show different results because *S.* Enteritidis is more successful at persistently colonising the internal organs of poultry compared with other serotypes.

Housing poultry in conventional cages was identified as a risk factor for *Salmonella* contamination in the 2011 Risk Profile. The move away from conventional cages to enriched cages or other production systems has prompted further studies to better predict if and how *Salmonella* contamination of layer facilities, flocks or eggs will change. However, investigations into the effects of different egg production processes (conventional and enriched cages, barn, free-range) on the prevalence of *Salmonella* in layers and eggs continue to yield conflicting and inconsistent results (Whiley and Ross, 2015).

Two USA studies found no significant difference in the prevalence of *Salmonella* in environmental samples and eggs taken from flocks housed in conventional cage, enriched, barn and/or free-range environments (Jones *et al.*, 2012; Jones *et al.*, 2015). Another USA study, using model environments mimicking conventional and free-range cages, reported a higher prevalence of *Salmonella* on eggs collected from hens kept in free-range environments (2.36%; 5/212) compared with eggs from conventionally caged hens (0/212) (Parisi *et al.*, 2015). It was suggested that hens have more contact with eggs after laying in free-range environments, and this increases the potential for microbiological contamination on the egg shell surface. This disagrees with an older European Food Safety Authority (EFSA) report that found cage production was associated with a higher risk of eggs being positive for *Salmonella* than other laying hen production systems, and pointed out that cage production was characterised by larger flock sizes, which are a risk factor for *Salmonella* contamination (EFSA, 2007). Cage type and flock size are probably both important risk factors for egg

contamination, and it has been claimed that the ultimate risk factor in these systems is the quality of flock management.[14]

In terms of prevalence among layers, a study comparing the ileal and caecal microbiota of hens kept in different caging systems found that the type of cage did not affect the pattern of colonisation and excretion of an *S.* Enteritidis inoculum (Nordentoft *et al.*, 2011). Another study of hens inoculated with *S.* Enteritidis found that hens living in conventional cages were more susceptible to intestinal colonisation (as measured by faecal excretion of the inoculum) than those living in enriched cages (with perching and nesting areas), but the type of caging system had no significant effect on faecal shedding over time (Gast *et al.*, 2015).

As mentioned in the 2011 Risk Profile, contaminated feed and water can be sources of *Salmonella* on the farm (Dewaele *et al.*, 2012b). Open troughs of drinking water can become contaminated by litter, feed, vectors and faeces. 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* spp. (Rivas, 2015). There is however, 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).

Based on industry data, the most common *Salmonella* serotype in 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 (Cressey *et al.*, 2011).

According to an investigation by the United States Food and Drug Administration (USFDA), the *Salmonella* outbreak at the Wright County Egg Farms in the US was possibly due to feed contamination as well as other environmental risk factors such as contaminated equipment and other surfaces within the farm (USCDC, 2010). The results of a Japanese survey showed that the primary isolation of *S*. Senftenberg was almost always from the feeds that then spread to the environment of the replacement pullet flocks and to other poultry farms (layers). Although, *S*. Senftenberg was not isolated from adult layer flocks or eggs, the characterisation of isolates and traceback results suggested that the feed (which was from a single source) played a major role in the introduction and transmission of *S*. Senftenberg into the poultry farms (Shirota *et al.*, 2012).

Salmonella might also be introduced to eggs during and after egg collection from contact with workers or surfaces, and this has been described in the 2011 Risk Profile. 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).

After eggs are collected, they generally undergo sorting, candling (crack detection), grading and packing. Extreme care must be taken during processing and handling to avoid cracks and damage to the egg shell surface which increases the risk of *Salmonella* invasion. Eggs that are cracked are often directed in the manufacture of egg products (e.g. pasteurised liquid egg), which are commonly used by the food service, hospitality and manufacturing industries.

[14] The *Salmonella* Initiative – stakeholder update (publication date not available). Provided by MPI, August 2015.

2.4 BEHAVIOUR OF *SALMONELLA* ON AND IN EGGS

Key findings

Experiments have demonstrated that the concentration of *Salmonella* on the shell of eggs decreases over time, but the rate of decrease is not predictable. Salmonellae are able to survive on eggs for one month or more at temperatures ranging from 4 to 26°C, when inoculated at high concentrations (5-7 log CFU). Survival is better under refrigeration and in the presence of faeces. Results suggest differences in survival between serotypes but this requires further study.

Non-Enteritidis serotypes can penetrate the egg shell and move into the albumen. Experiments studying the relationships between egg shell quality or egg washing and the ability of *Salmonella* to penetrate the shell are inconsistent, suggesting that the quality of the shell does not strongly influence *Salmonella* penetration. Lower temperatures slow the rate of penetration, but do not prevent it.

Scarce data on survival of *Salmonella* in the albumen suggests that survival is possible. *Salmonella* will grow in yolk or whole liquid egg depending on the temperature. Experimental data from the 2011 Risk Profile and this update show that *Salmonella* in yolk or whole liquid egg could grow at ≥7°C but will not grow at 4°C. Data on growth at temperatures between 4 and 7°C are needed.

It appears that pasteurisation regimes recommended for use in New Zealand would inactivate any *Salmonella* present in egg contents, but further validation would provide better assurance.

2.4.1 *Salmonella* behaviour on the surface of eggs

The 2011 Risk Profile reported that *Salmonella* only survived for a few days on the shell surface of clean eggs (where moisture and nutrients are low), but survival was better at low temperatures and high relative humidity, and in the presence of faecal matter. TABLE 1 shows the results from recent studies investigating *Salmonella* survival on egg shells, which changes some of the findings of the 2011 Risk Profile:

- *Salmonella* were able to survive on the surface of the eggs for several weeks (10 weeks in the study by Lublin *et al*. (2015)), although the number of cells put on the eggs was high (5-7 $\log_{10}$ CFU/egg);
- In one study comparing the effect of relative humidity, *Salmonella* survived better at a low relative humidity (43%) compared with a high relative humidity (85%); and
- Studies comparing survival at different temperatures showed inconsistent results.

Regarding storage temperature, the results by Park *et al.* (2015) showed that a cocktail of *Salmonella* serotypes survived better on the shells of whole, unwashed eggs at 4°C and 12°C compared with 22°C. The study by Lublin *et al*. (2015) also found survival of *S.* Infantis was better at 6°C compared with 26°C, particularly during the first two weeks (the relative humidity was similar at both temperatures). The work by Pasquali *et al.* (2016) indicated differences between serotypes, whereby *S.* Enteritidis survived better at 8 and 20°C compared with 4°C, and *S.* Typhimurium survived better at 4°C than the higher temperatures. However, their data were accompanied by large confidence intervals, and under some conditions there was no clear pattern of survival. Reasons for this are unclear but may be in part due to different levels of cuticle damage between eggs caused when they washed and sanitised the eggs before

applying the inoculum.[15] Finally, the study of McAuley *et al.* (2015) suggests that survival might be better at 22°C compared with 4°C, or at least similar, however the effect of relative humidity was not controlled in these experiments; this was lower at 22°C (38-55%, compared with 88-100% at 4°C).

An additional two Australian studies found that lower temperatures improved survival of *Salmonella* on the surface of eggs, but the storage temperatures compared were 20°C and 37°C over a period of 21 days, i.e. refrigeration was not examined (Gole *et al.*, 2014b; Gole *et al.*, 2014c). These studies found no significant difference in survival on washed or unwashed eggs, but survival differed between serotypes (serotypes Typhimurium, Singapore, Adelaide, Worthington and Livingstone were tested separately in these studies). The washing and sanitising was highly controlled in these studies.[16]

It is possible that the reduction in the concentration of *Salmonella* on the shell surface over time is partly due to cells migrating into the egg, but the extent of this effect is not established. Shell penetration is possible (see Section 2.4.2), but the available evidence supports cell death as the dominant process rather than cell migration. For example, Lublin *et al.* (2015) found the concentration of *S.* Infantis on the outside of whole eggs reduced by approximately 2 $\log_{10}$ CFU/g after two weeks at 26°C, but did not measure any *S.* Infantis in the egg contents.

The study by Park *et al*. (2015) confirmed that the presence of faeces enhances survival. Another study also evaluated survival in the presence of faeces (eggs were dip-inoculated into a solution containing 2% w/v faeces and 7 $\log_{10}$ CFU/ml *Salmonella*), and while enumeration was not consistently possible, *Salmonella* were detected by enrichment after approximately 50 days storage at 20°C and 80% or 90% relative humidity (Botey-Salo *et al.*, 2012).

The results from these studies show that it is difficult to predict the behaviour of *Salmonella* on the surface of eggs, but it can be assumed *Salmonella* can survive for several weeks. More reliable studies show that the concentration decreases over time and the rate of decrease is slower under cooler temperatures. Studies using lower concentrations of inoculum and focussing on non-Enteritidis serotypes are required, preferably with storage under conditions aligned with what eggs would be subjected to in the New Zealand food chain.

[15] Eggs were washed in water (method and time not specified) then in 70% ethanol for 30 minutes.
[16] Hydroxide and hypochlorite solutions in a mechanical washer (total wash time 68 seconds).

TABLE 1 Behaviour of *Salmonella* on the shell surface of eggs (studies published since 2011)

SEROTYPE	INOCULUM (log)	STORAGE CONDITIONS	CHANGE IN CONCENTRATION (log_{10} CFU/g)[1]	REFERENCE
Infantis	5.7 (in peptone)	5.5°C, 10 weeks 25.5°C, 10 weeks	↓ 2.1*[2] ↓ 1.5*[2]	(Lublin *et al.*, 2015)
Enteritidis (3 strains)	7.0 (in peptone)	7°C, 4 weeks	↓ 1.4	(Jin *et al.*, 2013)
Enteritidis	5 (in saline)	4°C, 4 weeks 8°C, 4 weeks 20°C, 4 weeks	↓ 4.0 ↓ 2.5 ↓ 3.0	(Pasquali *et al.*, 2016)
Typhimurium	5 (in saline)	4°C, 4 weeks 8°C, 4 weeks 20°C, 4 weeks	NC ↓ 2.0 ↓ 2.0	(Pasquali *et al.*, 2016)
Tennessee	5 (in saline)	4°C, 4 weeks 8°C, 4 weeks 20°C, 4 weeks	NC ↓ 1.0 NC	(Pasquali *et al.*, 2016)
Typhimurium	6 (in peptone)	4°C, 4 weeks 22°C, 4 weeks	↓ ND[3] ↓ ND[3]	(McAuley *et al.*, 2015)
Sofia	6 (in peptone)	4°C, 4 weeks 22°C, 4 weeks	↓ ND[3] ↓ ND[3]	(McAuley *et al.*, 2015)
Cocktail: Enteritidis, Typhimurium, Heidelberg, Hartford, Newport	5.6 (in saline)	85% relative humidity: 4°C, 3 weeks 12°C, 3 weeks 25°C, 3 weeks	 ↓ >5.6 ↓ >5.6 ↓ ND	(Park *et al.*, 2015)
Cocktail: Enteritidis, Typhimurium, Heidelberg, Hartford, Newport	6.0 (in saline + sterile faeces)	85% relative humidity: 4°C, 3 weeks 12°C, 3 weeks 25°C, 3 weeks	 ↓ 1.3 ↓ 1.4 ↓ 3.5	(Park *et al.*, 2015)
Cocktail: Enteritidis, Typhimurium, Heidelberg, Hartford, Newport	5.6 (in saline)	43% relative humidity: 4°C, 3 weeks 12°C, 3 weeks 25°C, 3 weeks	 ↓ 1.6 ↓ 2.1 ↓ 4.5	(Park *et al.*, 2015)

SEROTYPE	INOCULUM (log)	STORAGE CONDITIONS	CHANGE IN CONCENTRATION (log_{10} CFU/g)[1]	REFERENCE
Cocktail: Enteritidis, Typhimurium, Heidelberg, Hartford, Newport	6.0 (in saline + sterile faeces)	43% relative humidity: 4°C, 3 weeks 12°C, 3 weeks 25°C, 3 weeks	 ↓ 0.6 ↓ 1.0 ↓ 1.5	(Park *et al.*, 2015)

[1] ↓ = decreased by >0.5 log; ↑ = increased by >0.5 log; NC = no change (change ≤0.5 log); ND, not detected; * = inoculum also isolated from egg contents (internalisation). Data are reported from text, if provided, or estimated from graphs.

[2] By 2 weeks the concentration had decreased by approximately 2 log_{10} CFU/g at 26°C, but by <1 log_{10} CFU/g at 6°C. Thereafter the concentrations were similar between the two temperatures and did not vary significantly with time. The prevalence at 26°C also decreased from 100% at day 0 to 30% eggs positive by 2 weeks. At 6°C the prevalence was 90% at 2 weeks.

[3] *S.* Sofia: Detected at two weeks when stored at 4 and 22°C, but not at four weeks. *S.* Typhimurium: Not detected at one week at 4°C, not detected at two weeks at 22°C. Note the RH was 88-100% at 4°C, and was 35-55% at 22°C.

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

The 2011 Risk Profile reported that *Salmonella* can penetrate the egg shell 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 (e.g. how the egg is handled, the external conditions, the presence of faeces; see Section 2.3.1). The document also reported that refrigeration temperatures appear to reduce the ability of *Salmonella* to penetrate the egg shell, but these lower temperatures can also enhance penetration if eggs were previously stored at high temperatures and rapidly cooled.

Recent studies of egg shell 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 on 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 egg shells 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 egg shells of washed and unwashed eggs (Gole *et al.*, 2014b; Gole *et al.*, 2014c; Samiullah *et al.*, 2013). 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 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 carried out at 20 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 at 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 number 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).

Moreover, a review of older studies suggests that condensation on the eggs may increase *Salmonella* penetration of the shell (Martelli and Davies, 2012), but no recent studies were located to provide further evidence. The relationship between environmental temperature, relative humidity and egg shell temperature affects the development of condensation, and the right conditions for condensation are most likely to be found during cold chain distribution.

2.4.3 *Salmonella* behaviour inside whole eggs and liquid eggs

A number of intrinsic factors are present to inhibit or prevent *Salmonella* growth in the albumen, as described in the previous Risk Profile (Lake *et al.*, 2011). Slow growth may occur, although experimental results are difficult to interpret (Zhang *et al.*, 2011). Nevertheless, some *Salmonella* will persist in the albumen. Growth of any *Salmonella* that penetrate the defences and enter the albumen thus depends on their ability to reach the nutritious yolk, or else for the yolk nutrients to reach them.

Migration through the albumen and penetration of the vitelline membrane has been reported for *S.* Enteritidis (see 2011 Risk Profile). Refrigeration helps to reduce this migration and the growth rate (Gast *et al.*, 2013). Yolk can also be released as the vitelline membrane degrades over time, a process which is enhanced with increasing temperature (Whiting *et al.*, 2000).

In terms of growth in whole, intact eggs, the available information show that *S*. Typhimurium, *S.* Sofia and *S*. Enteritidis are able to survive (and possibly grow) in the albumen and either move to the yolk or encounter yolk as the vitelline membrane breaks down. The rate of bacterial growth is determined by storage temperature. There are insufficient data to inform the ability of other serotypes to survive in and migrate across the albumen, but it can be assumed that any serotype of *Salmonella* will grow in the yolk.

Experimental data assembled in the 2011 Risk Profile 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. Four studies published since 2011 were located that measured *Salmonella* growth in whole eggs or egg contents (Table 2, plus (McAuley *et al.*, 2015) and (Jakočiūnė *et al.*, 2014)). These studies support the earlier findings. Two studied non-Enteritidis serotypes (Lublin *et al.*, 2015; McAuley *et al.*, 2015) but in Lublin *et al.* (2015) the inocula were injected into the yolk so this study does not inform whether the serotype is able to migrate from the albumen to the yolk.

In the study of McAuley *et al.* (2015), the growth of two strains of *S.* Typhimurium and one strain of *S.* Sofia were separately monitored in unpasteurised liquid whole egg, liquid yolk or liquid albumen, 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 higher temperatures increased the growth rate. The growth rate in egg white (albumen) was minimal at all temperatures (combined mean 0.060 $\log_{10}$ CFU/ml/h at 37°C) for the period measured (up to 35 days). In egg yolk and whole egg respectively at the same temperature, the combined growth rates were 0.842 and 0.612 $\log_{10}$ CFU/ml/h. 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.

The study by Jakočiūnė et al. (2014) 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 and 50-58°C (Jakočiūnė *et al.*, 2014). Under the cooler temperatures, without added salt and at pH 7, the model predicted that *S*. Enteritidis can grow at temperatures above approximately 3°C (very slowly, 0.01 divisions/hour). The number of cells decreased 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.

Yolk Membrane Time (YMT) is a measure developed for a quantitative process model to assist risk assessment for *Salmonella* in eggs (Whiting *et al.*, 2000). It represents the number of days at a given temperature before eggs can support growth of *Salmonella*, i.e. it is a measure of the time- and temperature-dependent reduction in intrinsic defences to bacterial growth (Whiting *et al.*, 2000).[17] In risk assessments it is assumed that no growth is possible before YMT has been exceeded (Thomas *et al.*, 2006). Calculation of YMT assumes that if growth is observed in more than 20% of eggs in the experiments, then growth is possible. Growth was defined as more than 4 $\log_{10}$ *S*. Enteritidis per egg from an inoculum of 500 cells into the albumen. The 20% value is arbitrary (initially based on growth in 2/10 eggs) but allows for differences between individual eggs (Food Standards Australia New Zealand, 2009; Ministry for Primary Industries (MPI), 2015). This model is further discussed in Section 5.2.3.

[17] Note that other publications have referred to this as "yolk membrane breakdown time" or "yolk mean time".

TABLE 2 Behaviour of *Salmonella* in whole eggs or egg contents (studies published since 2011)

LOCATION ON EGG	SEROTYPE	INOCULUM	STORAGE CONDITIONS	CHANGE IN CONCENTRATION (log_{10} CFU/g)[1]	REFERENCE
In shell: Injected into yolk	Infantis	3.7 log_{10} CFU (in peptone)	5.5°C, 10 weeks 25.5°C, 10 weeks	↓<1.0 ↑ 3-4	(Lublin *et al.*, 2015)
Liquid whole egg (pasteurised)	Enteritidis	3.2 log_{10} CFU/g (in saline)	8°C, 30 days 12°C, 10 days 16°C, 5 days 20°C, 2.5 days	↑ 5.8 ↑ 6.5 ↑ 6.5 ↑ 6.5	(Sakha and Fujikawa, 2012)
Liquid whole egg (unpasteurised)[2]	Enteritidis	3.2 log_{10} CFU/g (in saline)	8°C, 20 days 12°C, 10 days 16°C, 5 days 20°C, 2.5 days	NC ↑ 0.8 ↑ 1.3 ↑ 1.5	(Sakha and Fujikawa, 2012)

[1] ↓ = decreased by >0.5 log; ↑ = increased by >0.5 log; NC = no change (change ≤0.5 log); ND, not detected. Data are reported from text, if provided, or estimated from graphs.
[2] Contained 7 log_{10} CFU/g of background microflora but no *Salmonella* spp.

2.4.4 *Salmonella* behaviour during pasteurisation and cooking

The 2011 Risk Profile included data on pasteurisation, D-times and survival during cooking for *Salmonella*. In summary:

- 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 0.31-0.69 minutes;
- Liquid albumin requires pasteurisation at lower temperatures (<60°C) to retain functionality, so D-values tend to be longer (e.g. $D_{52°C}$ 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).[18,19] Adherence to this Standard is only a regulatory requirement in Australia. The Standard specifies the following minimum temperature/times:

- Egg pulp (egg contents) 64°C/2.5 minutes.
- Liquid egg yolk, 60°C/3.5 minutes.
- Liquid egg white, 55°C/9.5 minutes.

A report from EFSA expressed a lack of certainty that the pasteurisation processes currently being used by industry effectively eradicated *Salmonella*, and recommend validation of the current industrial processes (EFSA Panel on Biological Hazards (BIOHAZ), 2010). In the USA, salmonellae are occasionally isolated from pasteurised egg products by food manufacturers or the USDA Food Safety and Inspection Service (FSIS), and may be present as a result of either pasteurisation-resistant bacteria or post-processing contamination (Gurtler et al., 2013). The above information 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 FSIS is responsible for regulating egg products in the USA, and requires liquid whole egg to be pasteurised at 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, Table 1 [Code of Federal Regulations, 2009]) (Gurtler et al 2015). Under this treatment regime, 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. Subsequent studies to investigate whether heat resistance could be linked to phenotypic characteristics did not find consistent results. Overall these experiments showed that *Salmonella* could survive this pasteurisation regime if present in a high enough concentration, and provided evidence of inter-strain survival differences.

[18] http://www.foodsafety.govt.nz/industry/sectors/poultry-eggs/eggs/food-standards-code-requirements.htm (accessed 14 December 2015).
[19] https://www.comlaw.gov.au/Details/F2011L00860 (accessed 14 December 2015).

Three other studies have been published since 2011 that evaluated the USA pasteurisation time/temperature regimes. Two of these evaluated *S.* Enteritidis and *S.* Oranienberg 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.*, 2013; Gurtler *et al.*, 2011). The third study developed a model for inactivation of salmonellae in commercial liquid egg yolk, based on survival studies of three strains of *Salmonella* (three Enteritidis, one Oranienburg) shown to have higher heat resistance (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 both of the USA pasteurisation regimes for liquid egg yolk (60°C/6.2 min or 61.1°C/3.5 min) would reduce *Salmonella* by at least 6-log.

2.5 EXPOSURE ASSESSMENT

Key findings

There are no new surveys of *Salmonella* prevalence on or in eggs in New Zealand. There are no data on *Salmonella* prevalence in New Zealand layer flocks or layer farm environments.

There were no recalls of eggs or egg products issued in New Zealand between 2011 and 2015 for potential contamination with *Salmonella*.

Data on egg consumption from New Zealand nutrition surveys from 2002 and 2009 indicate that almost half of the population consume egg on any given day. Most servings of eggs are cooked but consumption of raw egg was reported. The data do not provide information on the extent of egg cooking (i.e. times/temperatures or appearance, e.g. “runny”, “soft boiled”).

Salmonella will not grow on egg shells, but it is assumed that *Salmonella* contaminating the shell of whole eggs can survive on the egg from the point of lay to the point of consumption in New Zealand. This assumption comes from experimental data showing *Salmonella* can survive for a month or more on whole egg shells and the detection of *Salmonella* on the shells of whole eggs sampled at retail in New Zealand.

Assuming penetration into the egg contents, there is potential for *Salmonella* growth in whole eggs where refrigeration conditions are not maintained. Experimental data show that serotypes present in New Zealand could penetrate the shell and will grow if they reach the yolk either through migration through the albumen or through breakdown of the vitelline membrane. Experimental data also indicate that growth is inhibited below 7°C but further studies are needed. Available information on the storage conditions for eggs indicate that eggs in retail display are rarely stored under controlled refrigeration but eggs are likely to be stored in the refrigerator in consumer homes.

2.5.1 New Zealand prevalence studies

There have been no recent 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). 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) (Wilson, 2007).

The absence of eggs with contaminated contents was not surprising given the low prevalence of external contamination (Ministry for Primary Industries (MPI), 2015).

To date, there are no available data on the prevalence of *Salmonella* amongst layer hens or flocks, or the layer farm environments in New Zealand.

2.5.2 Product recalls

No recalls were issued in New Zealand between January 2011 and October 2015 for eggs or egg products potentially contaminated with *Salmonella*.[20]

2.5.3 Food consumption: Eggs

Eggs are commonly consumed in New Zealand. TABLE 3 summarises data on egg consumption by adult (15+ years) and child (5-14 years) New Zealanders. These figures are based on analyses of data (Cressey, 2013; Cressey *et al.*, 2006) from the 2009 Adult Nutrition Survey (2009ANS) (University of Otago and Ministry of Health, 2011) and the 2002 National Children's Nutrition Survey (2002CNS) (Ministry of Health, 2003). The 2011 Risk Profile presented data from the 1997 National Nutrition Survey (1997NNS), but the analyses used to produce these data differed to those used to produce the data in TABLE 3, so the two data sets will not be compared.

TABLE 3 Consumption of eggs by adult (15+ years) and child (5-14 years) New Zealanders (national nutrition surveys)

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

The data in TABLE 3 show that eggs are commonly consumed by New Zealand adults and children, although children consume smaller amounts in each serving.

Further analysis of the 2009ANS data has provided the following information.

Approximately two-thirds of egg servings were for eggs as an ingredient of a recipe (e.g. 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%

[20] New Zealand food recalls are advertised at http://www.foodsmart.govt.nz/food-safety/recalls/latest-recalls/ (accessed 1 July 2015).

- Eggs, whole, poached 13%

A small number of servings (15 out of 1087, 1.4%) related to consumption of raw eggs. This is double the proportion reported in the 2011 Risk Profile from the 1997NNS (0.7%, 7/1031), but this finding may be an artefact of different analytical 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).

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 are very similar to those of the general population.

The World Health Organisation Global Environmental Monitoring System (GEMS)/Food cluster diets give a range of egg consumption from 1.3 g/person/day (Cluster G16) to 42.1 g/person/day (Cluster G11).[21] G16 includes countries from central Africa, while G11 includes Belgium and The Netherlands. New Zealand is included in cluster G10 with daily egg consumption of 39.1 g/person/day.

2.5.4 Potential for growth of *Salmonella* along the egg food chain

Survival and growth of *Salmonella* on and in 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 21 days where the storage/holding temperature may exceed 15ºC, and a date of 35 days if stored or held at 15°C or less (Section 5.2.2). Whole eggs displayed at retail are rarely stored under controlled refrigeration yet the best before dates often exceed 21 days.[22]

There is no recent information to indicate the proportions of whole eggs that are refrigerated or stored at room temperature in New Zealander's 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 a majority of New Zealand households. A recent USA 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). 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).

The available data indicate that survival on the shells of whole eggs varies between *Salmonella* serotypes, and it is difficult to predict, but *Salmonella* will not grow on clean egg shells. Studies with more reliable data suggest that lower temperatures enhance survival. At ambient temperatures (8-20°C) *Salmonella* numbers reduce on the shells of eggs but can survive for a month or more, at least under laboratory conditions. Data on *Salmonella* survival on whole egg shells under normal commercial, retail and household conditions are lacking, so it must be assumed that at least some *Salmonella* contaminating the egg shells can survive on the egg from the point of lay to the point of consumption. Moreover, the detection of *Salmonella* on eggs sampled at retail in New Zealand proves that external contamination can occur under New Zealand egg production conditions, and persist through to retail. Faecal contamination enhances survival.

It has been shown that a number of different *Salmonella* serotypes can penetrate the shells of eggs but some serotypes appear to survive poorly in the albumen. Growth in the albumen is

[21] https://extranet.who.int/sree/Reports?op=vs&path=/WHO_HQ_Reports/G7/PROD/EXT/GEMS_cluster_diets_2012

[22] As observed by ESR staff in Auckland, Hamilton, Wellington and Christchurch supermarkets, December 2015.

limited, but 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 (studies on growth at temperatures between 4 and 7°C are needed). 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.

Pasteurisation or cooking will inactivate *Salmonella*, but complete killing of all microorganisms present depends on the temperature and time of cooking and the initial concentration of *Salmonella*.

2.6 DATA ON *SALMONELLA* AND EGGS FROM OTHER COUNTRIES

See appendices A.3 and A.4 for the detail summarised in this section.

Key findings

Environmental surveys of layer farms in two Australian states detected *Salmonella* in 49% of the farms (*S.* Typhimurium in 19%). A variety of other *Salmonella* serotypes were isolated but not *S*. Enteritidis.

The prevalence of *Salmonella*-positive layer flocks in the EU is decreasing. In 2013 the prevalence of *Salmonella*-positive flocks was 2.6% and the prevalence of flocks with *S*. Enteritidis and/or *S*. Typhimurium was 1%.

Data from overseas surveys of *Salmonella* in or on eggs presented in the 2011 Risk Profile showed very few instances where the prevalence of *Salmonella* on the outside or inside of the egg exceeded 1%. Overseas surveys published since 2011 continue to show low *Salmonella* prevalence on and in eggs (<1%).

3. EVALUATION OF ADVERSE HEALTH EFFECTS

3.1 DISEASE CHARACTERISTICS

Key findings

Salmonellosis is a self-limited infection for most people, but can result in severe outcomes (including death) or long term chronic conditions.

Information regarding the disease characteristics of non-typhoidal salmonellosis is outlined in the 2011 Risk Profile (Lake *et al.*, 2011).

Briefly, salmonellosis often presents with symptoms of gastroenteritis or enterocolitis, with non-bloody diarrhoea, abdominal pain, vomiting, nausea and fever. The illness is often self-limiting and can affect anyone, but the young, old and immunocompromised are particularly at risk for more severe symptoms such as extra-intestinal infections or bacteraemia and may require hospital treatment with fluid and electrolyte replacement, or antibiotics in cases of prolonged carriage or excretion. Long term chronic conditions such as Reiter's syndrome, septic arthritis or septicaemia can develop in some cases.

Information presented in Section 2.1 explained how *Salmonella* serotypes can differ in their ability to infect humans. Differences in health outcomes relative to serotypes have been reported overseas (Jones *et al.*, 2008). Analysis of salmonellosis cases in New Zealand between 2000 and 2009 found hospitalisation to be more often reported for cases infected with the serotypes Typhimurium, Infantis, Virchow and Thompson (Adlam *et al.*, 2010).

3.2 DOSE RESPONSE

Key findings

One dose response model based on salmonellosis outbreaks predicted a 50% probability of illness when exposed to 10^4 *Salmonella* cells. A second model, based on outbreaks, sporadic cases and human volunteer feeding studies predicted a 50% probability of illness when exposed to 36.3 cells, although the confidence interval was high (95th percentile 0.69-1.26x10^7). There is no known safe level of exposure to *Salmonella*.

The ability of *Salmonella* to cause illness, reflected in its dose-response, depends on the serotype, host susceptibilities, the food matrix and the infectious 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/or extrapolation from animal trials. The dose-response data for *Salmonella* currently rely largely on outbreak data. Modelling approaches attempt to account for known sources of error and variability.

There has been no new dose-response information relating to *Salmonella* published since the 2011 Risk Profile. The most applicable model continues to be outbreak data outlined in a 2002 FAO/WHO report which was developed further to incorporate differences in host susceptibility, serotype infectivity and food matrix (Bollaerts *et al.*, 2008). The analyses by Bollaerts *et al*. (2008) included modelling dose response for *S.* Enteritidis with egg as the matrix. An initial simulated dose-response that includes all food matrices and serotypes estimates a 50% probability of illness when normal people are exposed to approximately 1000 cells, or when susceptible people are exposed to approximately 100 cells.

The estimates published by Teunis *et al*. (2010) are also applicable (Teunis *et al.*, 2010). This study used data from 35 salmonellosis outbreaks, three sporadic cases for which there was good dose information and two human volunteer feeding studies. The model predicted a 50% probability of illness when exposed to 36.3 cells (95th percentile 0.69-1.26x10^{7}) and a 1% probability of illness when exposed to 0.4 cells.

In developing the Australian quantitative risk assessment model for *Salmonella* in eggs, Thomas *et al*. (2006) re-evaluated the FAO/WHO outbreak data and developed a different dose-response equation based on low doses (<100 cells) and high doses (>10^{6} cells) (Thomas *et al.*, 2006). The predicted probability of illness was similar between the FAO/WHO model and the alternative dose-response model. At intermediate doses, the estimated probabilities of illness from the alternative dose-response model were less variable than the WHO/FAO model.

3.3 NEW ZEALAND HUMAN HEALTH SURVEILLANCE

Key findings

Eggs have been implicated as the vehicle of infection for salmonellosis outbreaks in New Zealand by strong epidemiological and/or laboratory evidence. For the overall period 2000-2014, there were six salmonellosis outbreaks where eggs were implicated with strong evidence; this represented 1.9% of the total salmonellosis outbreaks reported during this period and 3.6% of the total cases associated with those outbreaks. There have been no reported case control studies investigating eggs as a risk/protective factor for salmonellosis since the 2011 Risk Profile.

The incidence of salmonellosis appears to be slowly decreasing in New Zealand. No deaths associated with salmonellosis have been reported since 2009, but the hospitalisation rate has remained stable at 13-19% of salmonellosis cases each year since 2005. There does not appear to be any change in the reported number of salmonellosis outbreaks each year. Over the period 2006-2014 salmonellosis outbreaks represented <5% of all enteric outbreaks each year and <3% of cases associated with enteric outbreaks each year.

S. Typhimurium is the most frequently isolated serotype from human salmonellosis cases in New Zealand, followed by *S*. Enteritidis (45% and 12%, respectively, for period 2010-2014).

Antimicrobial resistance among *Salmonella* isolated from human, animal and environmental samples in New Zealand remains relatively low but the percentage of tested isolates fully susceptible to all 12 antimicrobials has decreased from 92% in 2010 to 86% in 2014.

Salmonellosis is a notifiable disease in New Zealand. Diagnostic laboratories in New Zealand routinely submit all *Salmonella* isolates to ESR's Enteric Reference Laboratory for further typing (Nicol *et al.*, 2010). Typing includes serotyping, phage typing and antimicrobial

susceptibility testing. The Enteric Reference laboratory only undertakes phage typing for the Typhimurium, Enteritidis and Typhi serotypes. Pulsed-Field Gel Electrophoresis (PFGE), which is considered the 'gold standard' for the subtyping of *Salmonella* (Wattiau *et al.*, 2011), is used for salmonellosis outbreak investigations (see Appendix A.1). Multiple-Locus Variable-number tandem repeat Analysis (MLVA) is also used if PFGE does not result in sufficient discriminatory power (Muriel Dufour, ESR Enteric Reference Laboratory, personal communication, 10 September 2015). These molecular methods are described further in Appendix A.1.

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

There were 5,379 cases of non-typhoidal salmonellosis reported between 2010 and 2014 (Section 3.3.2). These were not analysed for evidence of eggs as a vehicle of transmission because transmission vehicles are rarely identified for any sporadic cases. This is evidenced by an analysis of 15,040 cases of non-typhoid salmonellosis reported between 2000 and 2009, which did not identify any case reports where a food or drink was confirmed as the source of infection by laboratory testing (Adlam *et al.*, 2010). Also analysed in this study was a sample of 208 notified salmonellosis cases where one or more probable foods were reported. Of these 208 cases, consumption of eggs was reported for nine cases but other foods were also reported as being consumed by these cases.

The 2011 Risk Profile reported on the results of an analysis of 204 salmonellosis outbreaks between 2000 and 2009. Egg-containing foods were implicated by strong evidence in four outbreaks (4/204, 2%).[23] Between 2010 and 2014 there were 106 reported salmonellosis outbreaks (Section 3.3.3) and eggs were implicated in three of these. Two outbreaks were considered to have strong evidence for egg as the vehicle of infection:

- *S*. Typhimurium DT155 (10 confirmed cases, 11 probable cases, 44 people exposed): Chocolate mousse cake made with raw egg served at a café/delicatessen. One batch of mousse was used in two cakes, which were consumed by three groups. Illnesses were reported in each group.
- *S*. Infantis (10 confirmed cases): Boiled egg and ham sandwich served at a café/bakery. *Salmonella* was isolated from the food.

Spanish cream made with raw eggs was implicated in the third outbreak. For the overall period 2000-2014, the six outbreaks with strong evidence represented 1.9% (6/310) of the total salmonellosis outbreaks reported and 3.6% (71/1966) of the total cases (confirmed and probable) associated with those outbreaks.[24]

There have been no new case control studies evaluating eggs as a risk factor for salmonellosis.

3.3.2 Salmonellosis in New Zealand

The 2011 Risk Profile showed that the annual rate of salmonellosis was >30 per 100,000 between 2005 and 2008, and the rate fell to 26.2 per 100,000 in the years 2009 and 2010. This lower reported rate has continued and the incidence of salmonellosis appears to be slowly decreasing in New Zealand (FIGURE 3, TABLE 4).

[23] *Salmonella* isolated from the food (2 outbreaks) or a food handler (2 outbreaks). See Adlam *et al.* (2010) for further information.
[24] 2000-2009: Total 204 outbreaks, 1426 cases. 2010-2014: Total 106 outbreaks, 540 cases (Section 3.3.3).

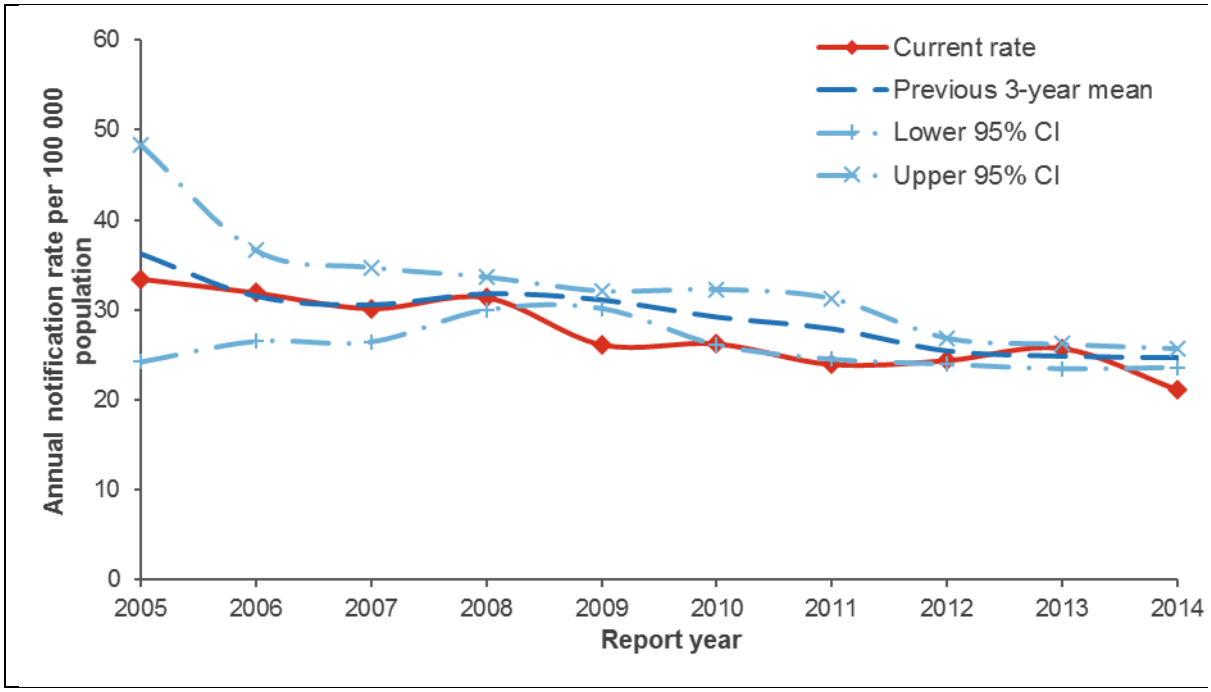

FIGURE 3: Salmonellosis notification rate by year, 2005–2014*

* Graph reproduced from Horn *et al.* (2015).

A study of the epidemiology of human salmonellosis in New Zealand, analysing data from 1997 to 2008, found (Lal *et al.*, 2012):

- The incidence was about twice a high in summer as in winter;
- Rural areas had higher rates than urban areas (particularly areas in the lower half of the South Island) and there was a distinct spring peak in rural areas;
- Incidence was highest in the 0-4 year age group; and
- Hospitalisation rates were higher for people living in more deprived areas or identifying themselves as Maori or Pacific Islander.

These findings have not changed over the period 2010 to 2014: There is a characteristic late summer peak and a winter trough, rates of salmonellosis vary throughout the country, but higher rates have been consistently seen in the lower half of the South Island, and age specific rates are highest for the <1 year age group (79.8 per 100,000 in 2014), and 1-4 year olds (68.0 per 100,000 in 2014).

While deaths associated with salmonellosis are rare and no deaths have been reported since 2009, between 13 and 19% of salmonellosis cases each year are hospitalised (TABLE 4).

TABLE 4: Notified cases of salmonellosis in New Zealand

YEAR	NUMBER OF CASES	RATE PER 100,000 POPULATION	HOSPITALISATION OF CASES (%)[1]	NUMBER OF CASES WHO DIED[1]	REFERENCES
2005	1383	33.7	142/1134 (13)	1/1383 (0.07)	(ESR, 2006)
2006	1335	31.9	148/1111 (13)	1/1335 (0.07)	(ESR, 2007)
2007	1274	30.1	110/833 (13)	1/1274 (0.07)	(ESR, 2008)
2008	1346	31.5	123/896 (14)	1/1346 (0.07)	(ESR, 2009)
2009	1129	26.2	134/716 (19)	1/1129 (0.09)	(ESR, 2010)
2010	1146	26.2	136/763 (18)	0/1146 (0)	(ESR, 2011a)
2011	1055	24.0	95/707 (13)	0/1055 (0)	(ESR, 2012a)
2012	1081	24.5	142/741 (19)	0/1081 (0)	(ESR, 2013a)
2013	1143	25.6	129/763 (17)	0/1143 (0)	(ESR, 2014a)
2014	954	21.2	104/630 (17)	0/954 (0)	(ESR, 2015a)

[1] The denominator is the number of cases for which the information about hospitalisation or death is known.

3.3.3 Reported New Zealand outbreaks

The 2011 Risk Profile reported data on salmonellosis outbreaks between 2005 and 2010. During this period, salmonellosis outbreaks represented <5% of all enteric outbreaks each year and <3% of cases associated with enteric outbreaks each year (except for 2005). This continues to be observed (TABLE 5). As a proportion of enteric outbreaks or cases, salmonellosis makes a small contribution; the outbreak data are dominated by reported outbreaks of norovirus.

While the reported rate of sporadic salmonellosis cases has decreased since 2009 (Section 3.3.2), there does not appear to be any change in the reported number of salmonellosis outbreaks. The three foodborne outbreaks where eggs were implicated have been discussed in more detail in Section 3.3.1.

TABLE 5: Reported salmonellosis outbreaks in New Zealand and information on those reported as foodborne (2010-2014)

YEAR	No. SALMONELLOSIS OUTBREAKS (% ALL REPORTED ENTERIC OUTBREAKS)	No. CASES ASSOCIATED WITH SALMONELLOSIS OUTBREAKS (% ALL CASES ASSOCIATED WITH ENTERIC OUTBREAKS)	No. SALMONELLOSIS OUTBREAKS REPORTED AS FOODBORNE (NUMBER OF CASES)[1]	No. FOODBORNE SALMONELLOSIS OUTBREAKS WHERE EGGS IMPLICATED (FOOD IMPLICATED)	REF[2]
2010	23 (4.0)	100 (1.6)	10 (56)	2 Chocolate mousse cake Spanish cream with uncooked eggs	*a*
2011	15 (2.7)	77 (1.1)	8 (42)	0	*b*
2012	27 (4.1)	149 (1.6)	11 (100)	0	*c*
2013	18 (2.9)	98 (1.4)	9 (45)	1 Boiled egg and ham sandwich	*d*
2014	23 (2.8)	116 (0.8)	7 (44)	0	*e*

[1] An outbreak is classed as foodborne if food was recorded as one of the likely modes of transmission applicable to the outbreak. Other modes of transmission may also be reported.

[2] References:

a (ESR, 2011b; Lim *et al.*, 2011)
b (ESR, 2012b; Lim *et al.*, 2012a)
c (ESR, 2013b; Lopez *et al.*, 2013)
d (ESR, 2014b; Horn *et al.*, 2014)
e (ESR, 2015b; Horn *et al.*, 2015)

3.3.4 Serotypes causing disease in New Zealand

The 2011 Risk Profile reported that of 11,554 serotyped isolates from human salmonellosis cases in New Zealand during the period 2000-2009, 58% were *S.* Typhimurium (mostly DT 160) and 9% were *S.* Enteritidis.

The serotype was available for 5,326 human salmonellosis cases reported for the period 2010-2014 (Horn *et al.*, 2015). *S.* Typhimurium and *S.* Enteritidis were still the most frequently isolated serotypes (45% and 12%, respectively). When considering serotype and phage type, *S.* Typhimurium DT56 variant was most frequently reported (8% of isolates),[25] followed by *S.* Typhimurium DT160 (6%) (TABLE 6). The incidence of *S.* Typhimurium DT160 has been declining in the last five years and a peak of *S.* Typhimurium DT56 variants was recorded in 2013 (Horn *et al.*, 2015). The 22 serotypes causing 50 or more salmonellosis cases over the period 2010 and 2014 (TABLE 6) together caused 65% (3,450) of the 5,326 cases for which serotypes were obtained.

[25] Prior to 2013, *S.* Typhimurium DT56 variant was reported as *S.* Typhimurium RDNC-May 06 (Horn *et al*. 2015).

TABLE 6: *Salmonella* serotypes that caused 50 or more cases during the period 2010 to 2014 – peak occurrence and total cases[1]

SALMONELLA SEROTYPE	PEAK OCCURRENCE[2]					TOTAL CASES 2010-2014
	2010	2011	2012	2013	2014	
Typhimurium DT56 variant	+	+	+	+	+	425
Typhimurium DT160	+	+	+	+		327
Infantis	+	+	+	+	+	297
Enteritidis PT11		+	+			223
Typhimurium DT135						222
Typhimurium DT101	+	+				213
Brandenburg				+		202
Typhimurium DT1		+				177
Saintpaul						161
Stanley						143
Salmonella enterica 4, [5],12: i: -						129
Typhimurium DT12a						124
Weltevreden						122
Typhimurium DT156						111
Typhimurium DT42						97
Typhimurium DT9						91
Mississippi						75
Virchow						71
Agona						69
Typhimurium DT23						60
Montevideo						58
Typhimurium DT74						53

[1] Data are from reports available from https://surv.esr.cri.nz/enteric_reference/human_salmonella.php (accessed 30 November 2015) and from (Horn *et al.*, 2015).

[2] + denotes where number of cases in a single year exceeded 50. Note that this differs from the approach used in Table 10 of the 2011 Risk Profile.

3.3.5 Antimicrobial resistance of New Zealand *Salmonella* strains

ESR's Antibiotic Reference Laboratory tests the antimicrobial resistance of approximately 20% of all human and non-human *Salmonella* isolates received for typing, along with all *S.* Typhimurium phage types that are internationally recognised as being multiresistant. The most recent report is for 2014 which states: "Antimicrobial resistance among *Salmonella* remains relatively low, with 85.5% (79.5% of human isolates and 94.3% of food/animal/environmental isolates) fully susceptible to all 12 antimicrobials."[26] The percentage of tested isolates fully susceptible to all 12 antimicrobials has decreased from 92% in 2010 to 86% in 2014. Further details are given in Appendix A.2.

[26] https://surv.esr.cri.nz/PDF_surveillance/Antimicrobial/SAL/SAL_2014.pdf (accessed 30 November 2015).

3.3.6 Case-control studies and risk factors

To date, seven case control studies of salmonellosis have been conducted in New Zealand. Four of these (NZFSA Science Group, 2007; Thornley *et al.*, 2003) examined eggs as a potential risk factor for salmonellosis. In one, consumption of eggs was a significant risk factor for an outbreak of *S.* Mbandaka infection, and in another consumption of eggs was protective (this study was focussed on *S.* Brandenburg, which is associated with sheep). These case-studies are outlined in the 2004 and 2011 Risk Profiles.

No additional case-control studies of New Zealand salmonellosis cases have been conducted since the 2011 Risk Profile. Two case series investigations were carried out in response to outbreaks of salmonellosis (McCallum *et al.*, 2013; Paine *et al.*, 2014). Eggs were not implicated in either of these outbreaks and the causative foods were determined to be tahini and raw wheaten flour.

3.4 SALMONELLOSIS OVERSEAS

See Appendices B.1-B.5 for the detail summarised in this section.

Key findings

New Zealand's salmonellosis rate remains elevated above those of the EU (as a whole), USA and Canada. Australia's salmonellosis rate continues to be higher than that of New Zealand. *S.* Enteritidis is the dominant serotype isolated from salmonellosis cases in the EU and North America, followed by *S.* Typhimurium.

The rate of salmonellosis in the EU is decreasing and this has been attributed to greater controls implemented in the broiler and layer production chains, targeting *Salmonella*.

In Australia, the rate of salmonellosis has increased significantly over the last 10 years. Increased *S.* Typhimurium and *S.* Enteritidis infections have both been reported. Like New Zealand, cases with *S.* Enteritidis infection most often reported overseas travel during the incubation period. There has also been an increase in reported salmonellosis outbreaks in Australia, with several attributed to consumption of raw or undercooked eggs.

Outbreaks caused by eggs contaminated with non-Enteritidis serotypes have been reported overseas. Some of these outbreaks have been traced back to the egg producing farm. Not all of the reports specified whether the eggs were undercooked or raw. Cross-contamination from the raw eggs is also possible.

Analyses of outbreak data from the USA and Europe support eggs and egg products as vehicles for *Salmonella* infection. Two recent case control studies also found an association between salmonellosis and eggs, although one only found an association between salmonellosis and handling eggs without subsequent hand washing. Attribution studies in the EU, USA and South Australia have determined layer hens and eggs are important contributors to the overall burden of human salmonellosis.

4. EVALUATION OF RISK

4.1 RISK ASSESSMENTS

Key findings

There are no risk assessments considering *Salmonella* in or on eggs in New Zealand.

An Australian quantitative risk assessment considering *Salmonella* contamination of eggs found the prevalence of *Salmonella*-contaminated eggs and storage temperature were important factors affecting estimated salmonellosis cases.

4.1.1 New Zealand risk assessments

There are no risk assessments considering *Salmonella* in or on eggs in New Zealand, although the recent MPI publication considering horizontal transfer and growth of *Salmonella* in chicken eggs includes some aspects of a quantitative risk assessment. Relevant information is cited elsewhere in this Risk Profile.

4.1.2 Risk assessments from other countries

The following is a summary of Appendix B.6.

The quantitative risk assessment published by FSANZ for Australia is most relevant to New Zealand because it assumed that eggs were not internally contaminated at the point of lay. This was completed in 2009 but was not available at the time the 2011 Risk Profile was being prepared. The model found temperature was a key determinant of *Salmonella* growth in eggs; the model estimated that, in the absence of any cooking, storage of eggs under temperatures permitting *Salmonella* growth would cause nine times more salmonellosis cases than if the eggs were stored under conditions preventing *Salmonella* growth. Preventing contamination of eggs on-farm was also shown to be important, as the risk of illness from raw eggs stored under conditions permitting *Salmonella* growth proportionately reduced as the prevalence of contaminated eggs reduced.[27]

Risk assessments have also been published by EFSA and Health Canada, but these considered shell eggs internally contaminated with *S.* Enteritidis, so are of less relevance to the New Zealand situation.

[27] In January 2016 FSANZ released a statement in response to public debate over refrigeration of eggs during retail storage, see http://www.foodstandards.gov.au/media/Pages/Statement-on-egg-food-safety-.aspx (accessed 15 March 2016).

4.2 EVALUATION OF RISK FOR NEW ZEALAND

Key findings

From available data, the public health risk from *Salmonella* in or on eggs consumed in New Zealand has not changed since the 2011 Risk Profile, i.e. there is little evidence that transmission of *Salmonella* via eggs is a significant transmission route occurring in New Zealand.

However, there is evidence to show that whole, fresh eggs sold in New Zealand can be contaminated with *Salmonella* and this may be contributing to a small (but undefined) proportion of human illness.

There is not enough data to assess the risk from liquid or dried eggs.

4.2.1 Risk associated with eggs

RMQ1: Has the public health risk from *Salmonella* in or on eggs consumed in New Zealand changed since the 2011 Risk Profile?

The 2004 Risk Profile concluded "There is little evidence that transmission of *Salmonella* via eggs is a significant transmission route occurring in New Zealand."

The 2011 Risk Profile concluded that "the risk of salmonellosis from consumption of eggs does not appear to have changed since the 2004 Risk Profile".

This was based on:

- The static incidence of reported illness; and
- The continued dominance of *S.* Typhimurium in reported cases rather than *S.* Enteritidis.

The 2011 Risk Profile also noted:

- The sparse epidemiological evidence linking eggs with salmonellosis;
- A prevalence of 1.8% for *Salmonella* on the shells of eggs (not detected in the contents);
- That externally contaminated eggs can contaminate foods, hand, utensils and surfaces;
- That migration of *Salmonella* from the shell surface to the contents is possible, but should be rare under refrigeration; and
- That periodic spikes of *Salmonella* contamination of eggs are the most likely pattern for prevalence.

With the exception of the incidence of reported illness (the rate of salmonellosis has decreased since 2011), the data collated in this update continue to support these points.

From available data, the public health risk from *Salmonella* in or on eggs consumed in New Zealand has not changed since the 2011 Risk Profile, i.e. there is little evidence that transmission of *Salmonella* via eggs is a major transmission route occurring in New Zealand. However, transmission of *Salmonella* via eggs appears to be a minor transmission route in New Zealand, based on the following information.

The available data indicate that eggs produced in New Zealand can potentially be externally contaminated by *Salmonella*:

- *Salmonella* have been isolated from the shells of whole, fresh eggs purchased at retail in New Zealand (1.8% in the most recent 2007 survey).

- *Salmonella* numbers decrease on the shell of whole, fresh eggs, but salmonellae have been shown to survive for a month or more under experimental conditions (indicating capacity to survive from point-of-lay to point-of-consumption).
- A recent survey in Australia found the prevalence of *Salmonella* on whole, fresh eggs to be low (0.5%), yet environmental surveys of layer farms in two Australian states detected *Salmonella* in 49% of the farms (*S.* Typhimurium in 19%), which indicates substantial potential for egg contamination (Cuttell *et al.*, 2015; NSW Food Authority, 2013a). While there are no data on *Salmonella* prevalence in New Zealand layer flocks or layer farm environments, the low prevalence of contamination in eggs does not preclude a high prevalence in flocks or farm environments.

Contamination of the contents of whole, fresh eggs by *Salmonella* is probably a rare event in New Zealand (particularly since *S.* Enteritidis is not endemic among New Zealand flocks). However, experimental evidence shows that internal contamination is possible for some non-Enteritidis serotypes, and from the limited information on egg storage temperatures, there is opportunity for *Salmonella* to multiply in the egg if present:

- *Salmonella* have not been isolated from egg contents in any New Zealand surveys (although this may be an artefact of sample size).
- *S.* Enteritidis has not been isolated from eggs in any New Zealand surveys. *S.* Typhimurium has been shown to colonise the reproductive organs of hens but, unlike *S.* Enteritidis, it is not certain how important vertical transmission is for this serotype.
- Experiments have demonstrated the ability of several non-Enteritidis serotypes to migrate across the egg shell into the contents and to survive in the albumen;
- Migration through the albumen to the yolk has only been proven for *S.* Enteritidis, but release of the yolk with breakdown of the vitelline membrane will support growth of *Salmonella* in the albumen.
- Breakdown of the vitelline membrane is accelerated with increasing temperature. Growth of *Salmonella* in the contents of eggs appears to be supported at temperatures of 7°C or above (data between 4 and 7°C are lacking). Data on temperature controls for eggs between lay and retail are unavailable. Based on the anecdotal information collected for this update, eggs at retail are rarely refrigerated in New Zealand. Older studies suggest eggs are often refrigerated in consumer homes but a small proportion of consumer refrigerators may be operating above 8°C.

There is evidence to show that eggs are a vehicle for salmonellosis in New Zealand:

- Salmonellosis outbreaks have been reported in New Zealand where there was strong evidence implicating eggs as the vehicle of infection (1.9% of the total salmonellosis outbreaks during the period 2000-2014 and 3.6% of the total cases associated with those outbreaks).
- While the most common serotype isolated for salmonellosis cases in New Zealand, *S.* Typhimurium, has not been isolated from surveys of eggs in New Zealand, *S.* Infantis was identified from all nine *Salmonella*-positive eggs in the 2007 survey. *S.* Infantis was identified from 6% of the human *Salmonella* isolates that were serotyped in 2014.

Data from national nutrition surveys indicate that eggs are consumed by almost half of New Zealanders each day. The risk of illness if *Salmonella* are present will be mitigated because the majority of egg servings are cooked and only a very small proportion of servings appear to be consumed raw. Some egg cooking processes will be insufficient to eliminate any *Salmonella* present.

Overseas studies have identified an increasing popularity of unprocessed home-made foods containing raw eggs such as mayonnaise, certain sauces and raw egg-based deserts like ice

cream, which increases the risk of salmonellosis (The OzFoodNet Working Group, 2015; Whiley and Ross, 2015). In Australia, the incidence of egg-associated salmonellosis has risen rapidly over the past five years and while the reason is not clear, a common element of many of these outbreaks is the consumption of raw or undercooked eggs, particularly in desserts and sauces, and also the use of dirty and/or cracked eggs.

Salmonella contaminating an egg shell could cause illness if introduced to other foods (pooled eggs or cross-contamination) or may pose a risk for the food handler (e.g. touching mouth after shelling eggs). Kitchen surfaces and utensils may become contaminated by raw contaminated eggs and cross-contaminate other foods. Slinko *et al*. (2009) described an outbreak of *S.* Typhimurium phage type 197 in a series of restaurants in Australia over a two-month period where cross-contamination from cracked and dirty eggs was an issue in both premises (Slinko *et al.*, 2009).

There are few New Zealand data on liquid eggs (whole, yolk, albumen) or dried egg use and consumption. Liquid products are available at retail in New Zealand in pasteurised and unpasteurised forms, and both products will also be used by food manufacturers or food service outlets. However, data for assessing risk are absent, e.g. the prevalence of *Salmonella* in these egg preparations, the times/temperatures for storage or drying or pasteurisation, the frequency and amount consumed.

4.2.2 Risks associated with other foods

Internationally, poultry is considered to be one of the most important food vehicles for *Salmonella.* Since 2011, a Risk Profile on *Salmonella* in poultry was updated (King *et al.*, 2011). This concluded that "the low risk from this food/hazard combination, as assessed by the 2004 Risk Profile, does not appear to have changed. On the basis of the reduced prevalence in *Salmonella* found on poultry carcassess by the NMD testing programme from 2005-2010, it could be argued that the risk has declined."

Other foods have also been associated with salmonellosis in New Zealand. *Salmonella* has been associated with ready-to-eat fresh produce (King *et al.*, 2015). A salmonellosis outbreak in 2008 was caused by flour contaminated with *S.* Typhimurium phage type 42 (McCallum *et al.*, 2013). There have been several outbreaks in New Zealand caused by *Salmonella* in tahini (Lake *et al.*, 2010; Paine *et al.*, 2014).

It may be that there is no dominant food vehicle for salmonellosis in New Zealand. The complex nature of the epidemiology of salmonellosis in New Zealand is underlined by a recent study that found differing patterns of disease at the serotype level (French *et al.*, 2011). Depending on the serotype, environmental, zoonotic or foodborne transmission may be more important.

4.3 THE BURDEN OF SALMONELLOSIS IN NEW ZEALAND

Key findings

Information in this Risk Profile signals that the burden of salmonellosis in New Zealand from eggs contaminated with *Salmonella* is likely to be a small proportion of the overall burden of salmonellosis in New Zealand.

4.3.1 Burden of disease from eggs contaminated with *Salmonella*

There is no estimate of the burden of disease caused by eggs contaminated with *Salmonella* in New Zealand. Based on the available information and the assessment of risk (Section 4.2.1) the burden of salmonellosis caused by exposure to eggs is likely to be a small proportion of the overall burden of salmonellosis in New Zealand.

4.3.2 Burden of disease from all salmonellosis

The latest update of the estimate of the burden of foodborne disease for New Zealand (Cressey and Lake, 2014) includes an estimate for foodborne salmonellosis of 74 disability adjusted life years (DALYs). This placed foodborne salmonellosis fifth on the list for foodborne disease burden (after campylobacteriosis, norovirus infection, perinatal listeriosis and STEC infection).

The New Zealand estimates of the burden of foodborne disease from salmonellosis do not subdivide the burden according to specific foods.

An expert elicitation carried out in 2013 derived an estimate for the proportion of salmonellosis in New Zealand that is due to foodborne transmission of 62.1% (95th percentile credible interval 35.2-86.4%, based on self-assessed performance weighting) (Cressey and Lake, 2013). The proportion of foodborne transmission due to eggs was not determined, but the proportion due to poultry was estimated to be 19.2% (95th percentile credible interval 3.0-56.5%).

A recent burden of foodborne illness study in The Netherlands estimated that the burden of disease due to foodborne salmonellosis was approximately 650 DALYs per year or 12 million Euros (Mangen *et al.*, 2015). Given that the population of The Netherlands is approximately four times the population of New Zealand, this suggests a greater per capita burden due to salmonellosis in The Netherlands than New Zealand. Based on cost of illness, foodborne salmonellosis accounted for the fifth greatest cost of illness in The Netherlands, after *Staphylococcus aureus* intoxication, *Clostridium perfringens* intoxication, campylobacteriosis and norovirus infection.

An Australian study estimated the annual burden of salmonellosis as 3856 DALYs (Gibney *et al.*, 2014). It should be noted that this included both foodborne and non-foodborne cases and included consideration of reactive arthritis and irritable bowel syndrome (IBS) as sequelae to gastroenteritis. The New Zealand study included reactive arthritis and inflammatory bowel disease, but not IBS, as sequelae to *Salmonella* infections (Cressey and Lake, 2014). IBS accounted for slightly more than one-third of the Australian DALY estimate for salmonellosis.

A US study used quality-adjusted life years (QALYs) and cost of illness (COI) to measure the burden of disease associated with 14 foodborne pathogens (Hoffmann *et al.*, 2012). Salmonellosis accounted for the highest mean QALY loss of any of the pathogens (16,782 QALYs), followed by campylobacteriosis, toxoplasmosis and listeriosis. COI gave a slightly different ranking, but the greatest cost was associated with salmonellosis ($3309.3 million), followed by toxoplasmosis, listeriosis and norovirus infection.

4.4 DATA GAPS

Key findings

There are data gaps relating to all points along the New Zealand food chain for eggs. The most important data gaps are information on the conditions under which eggs are held during storage, retail and in consumer homes, and the behaviour of non-Enteritidis serotypes on and in eggs.

The data gaps identified in the 2011 Risk Profile are still valid. These are:

- Representative sampling and testing for *Salmonella* in egg layer farm inputs (feed) and environment.

- Additional sampling for *Salmonella* in eggs during production and retail.
- Extensive typing and case follow-up of *Salmonella* isolates obtained from clinical, animal and food sources.
- Determine the potential of New Zealand *Salmonella* isolates to penetrate and grow in eggs during production and storage.
- Egg processing and retail handling in New Zealand.
- Egg storage and consumer handling practices in New Zealand.

With regard to the first point above, a baseline survey of *Salmonella* contamination in housing and eggs from different layer production systems in New Zealand (conventional, colony, barn, free-range) would provide information towards anticipating changes in contamination levels with the upcoming move away from conventional cages.

With regard to the final two points, information on egg storage conditions, handling and cooking (or not) is important for assessing risk. There are few data on the storage, use and consumption of liquid and dried eggs in New Zealand, and how *Salmonella* behaves in these products. Data on the ability of different *Salmonella* serotypes to grow in whole egg, yolk or whole liquid egg at temperatures between 4 and 7°C requires further study.

A quantitative risk assessment relevant to New Zealand conditions also requires data on the behaviour of non-Enteritidis serotypes on the surface of egg shells with storage under conditions aligned with what eggs would be subjected to in the New Zealand food chain.

5. CONTROLS

5.1 CURRENT CONTROL MEASURES

Key findings

MPI have implemented a Risk Management Strategy for reducing salmonellosis in New Zealand.

There is no regulatory requirement for *Salmonella* monitoring in New Zealand layer farms and/or packhouses. There are no microbiological standards for *Salmonella* on or in whole eggs but *Salmonella* must not be detected in five 25 g samples of pasteurised egg products or processed egg product.

Most layer farms must have a registered Risk Management Programme that includes some controls specific for managing *Salmonella*, and other more general controls that will help to manage *Salmonella*.

A new code of welfare for layer hens was introduced in 2012 which also includes general controls that will help manage *Salmonella* on layer farms. Conventional cages will be replaced by colony, barn or free-range cages by 2022 under this code.

Under the new *Food Act* 2014, manufacturers of processed egg products who do not operate under a Risk Management Programme will be required to operate under a Food Control Plan.

5.1.1 Risk Management Strategy

At the time of writing, the latest MPI Risk Management Strategy for *Salmonella* covered the period 2013-14.[28] The aim of the Strategy is to maintain the 30% reduction in the reported annual incidence of foodborne salmonellosis over the last five years and to support market access. The strategy focuses on gathering information about a range of potential sources of *Salmonella* (non-typhoidal strains) and continues to investigate high risk foods and handling processes.

The Strategy includes a summary of the situation regarding eggs:

"Most of the egg production and packing sector has been required to have RMPs from 2003-2004 to control hazards to human health, including *Salmonella*. Retail egg surveys (1994-2007) have shown an absence of internal contamination of eggs by *Salmonella*. Except for two foodborne outbreaks in 2010, specifically implicating *Salmonella* in eggs, no further significant foodborne disease has been associated with *Salmonella* and eggs over 2011-2012." One further outbreak with strong evidence implicating eggs has occurred since then.

RMPs are Risk Management Programmes (see below).

A recently-completed project from the work programme signalled in this Strategy was the literature review of the ability of *Salmonella* on egg shells to penetrate the shell and grow during storage for up to 35 day. This was published in 2015 and information from this has been cited throughout this Risk Profile (Ministry for Primary Industries (MPI), 2015).

[28] http://www.foodsafety.govt.nz/elibrary/industry/salmonella-strategy_2010-14.pdf (accessed 7 December 2015)

5.1.2 Relevant food controls

There is no regulatory requirement in New Zealand for managers of layer farms and/or packhouses to routinely monitor for *Salmonella* among the layer flocks or whole eggs, or the farm/packhouse environment. Some managers will undertake such monitoring voluntarily or to meet export conditions (Lisa Olsen, MPI, pers. comm.). Standard 1.6.1 of the Food Standards Code for Australia and New Zealand sets out microbiological standards for foods. There are no standards for *Salmonella* on or in whole eggs but *Salmonella* must not be detected in five 25 g samples of processed egg product (Food Standards Australia New Zealand, 2016).[29]

Most layer farms must have a registered Risk Management Programme (RMP) under the *Animal Products Act* 1999.[30] The RMP requires farm managers to have a whole flock health scheme to minimise the chance that layers are contaminated with *Salmonella*.[31] Some of the controls include requirements to specify how *Salmonella* will be controlled in feed and whether *Salmonella* vaccination is undertaken. Vaccination is not compulsory. The 2011 Risk Profile included information from a Technical Appendix to the Generic Code of Practice for Egg Production (published in 2002). This Code of Practice has been replaced by the RMP template and this template includes egg washing and storage requirements. The RMP template also includes requirements that will help to prevent *Salmonella*-contaminated eggs from reaching the consumer, e.g. in-shell eggs must be visibly clean, must be handled to minimise condensation and must undergo candling to ensure integrity, and dirty, cracked or floor eggs must be collected separately.

The 2011 Risk Profile mentioned the Animal Welfare (Layer Hens) Code of Welfare 2005, issued under the Animal Welfare Act 1999. A new version of this Code came into force on 7 December 2012, and a 2013 amendment was gazetted in December 2013 (National Animal Welfare Advisory Committee, 2012). The Animal Welfare (Layer Hens) Code of Welfare 2012 sets out the standards of care and management for layer hens in New Zealand.

The new Code is specific in its requirements of farmers and includes new detailed sections on colony farming, range management for free-range farming, natural animal behaviour, and the handling, catching and transport of layer hens and chicks. The Code also identified good stockmanship as the key to good welfare. The Code does not include any specific requirements for controlling *Salmonella* but some of the requirements would help prevent or control flock infection, e.g. manure removal under cages, prevention of induced moulting and handling methods that minimise stress. A particular feature of the new Code is that conventional cages will be phased out by the end of 2022. The 2013 amendment to the Code extended the timeframes for layer farms to replace any conventional cages, but the final date of 2022 remains unchanged.[32]

Secondary processors of eggs (i.e. those who manufacture processed egg products such as liquid or dried egg) can choose to operate under a RMP. The alternative is to operate under the *Food Act* 1981, either under the Food Hygiene Regulations 1974 or with a Food Safety Plan.[33] The *Food Act* 2014 will be fully in force by 1 March 2016 and will replace the *Food Act*

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[29] Schedule 27 can be viewed at: https://www.legislation.gov.au/Details/F2016C00200 (accessed 16 March 2016).
[30] http://www.foodsafety.govt.nz/industry/sectors/poultry-eggs/eggs/APA-requirements.htm (accessed 2 December 2015).
[31] The template and appendices are available from http://foodsafety.govt.nz/elibrary/industry/template-eggs/index.htm (accessed 14 December 2015).
[32] http://www.beehive.govt.nz/release/amendments-layer-hens-code-welfare (accessed 2 December 2015).
[33] http://www.foodsafety.govt.nz/industry/sectors/poultry-eggs/eggs/food-act-requirements.htm (accessed 17 December 2015).

1981 and, over time, replace the Food Hygiene Regulations 1974. Under the *Food Act* 2014, manufacturers of processed egg products (not operating under a RMP) will be required to operate under a Food Control Plan, and must transition to this plan by 30 June 2018. Those operating under a Food Safety Plan can continue under this plan until 28 February 2019.[34]

5.2 ADDITIONAL OPTIONS FOR RISK MANAGEMENT

Key findings

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

There are multiple interventions that can be applied on-farm, but prevention and control of *Salmonella* is best achieved through a comprehensive programme incorporating multiple controls. Vaccination is recommended in New Zealand but not compulsory. Feeding prebiotics and probiotics to hens has been shown to provide some protection against *Salmonella*. Environmental management includes controlling the food and water supply, biosecurity and pest management, and ensuring effective cleaning regimes are in place.

Maintaining refrigeration of eggs post-lay will control the growth of any *Salmonella* that might be present in the egg contents. Egg washing/sanitising is optional in New Zealand, but international opinion differs with respect to the effectiveness of this intervention in controlling *Salmonella*. Recent information suggests that pasteurisation regimes in use in New Zealand need further validation to ensure they achieve sufficient *Salmonella* reduction.

The information available on interventions is extensive and needs to be assessed for the applicability in the New Zealand context. A separate, more comprehensive review of the efficacy of intervention options relevant to New Zealand is recommended.

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

MPI has published a review that examined whether the number of salmonellosis cases attributed to eggs would increase if the shelf life of eggs were extended to 35 days, irrespective of temperature. MPI concluded that "it would appear prudent to maintain the current requirements for handling and storage of eggs". The New Zealand Risk Management Programme for eggs sets out temperature controls for eggs, which requires temperatures to be maintained at 15°C or below for eggs stored up to 35 days post-lay.

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

Environmental sampling at layer farms more efficiently and effectively detects the potential for *Salmonella* to contaminate eggs. An effective sampling regime will include both faeces and dust, and will maximise the number of samples taken. A separate study is recommended to understand the relationship (if any) between the results of environmental surveys of layer housing and the prevalence of *Salmonella* on eggs in New Zealand. Such a study could investigate the relationship between a *Salmonella*-positive flock and *Salmonella*-positive eggs. Mathematical modelling to predict the likely prevalence of *Salmonella*-positive eggs in New Zealand, given a prevalence of *Salmonella*-positive flocks would also inform shelf life considerations.

[34] https://www.mpi.govt.nz/food-safety/food-act-2014/food-control-plans/ (accessed 17 December 2015).

This section focuses on three RMQs:

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

Given the ability of *Salmonella* to survive on the egg shell for the period between production and retail sale, and the limited ability of cleaning to eliminate contamination (and possibly promote horizontal transmission), the emphasis is on preventing *Salmonella* contamination of eggs on-farm. Interventions in the areas of animal management and environmental management are introduced. It is not achievable to produce eggs guaranteed to be *Salmonella*-free (Whiley and Ross, 2015), so post-lay measures of temperature control and egg sanitising are also discussed.

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

A summary of information useful for considering egg shelf life in the context of the public health risk from *Salmonella*.

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

A discussion of options for gathering data on *Salmonella* prevalence in the layer industry.

5.2.1 On-farm controls

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

This section does not present a full evaluation of all potential on-farm controls for *Salmonella*, since this requires a separate, comprehensive review that should incorporate recent and older literature and should consider, for each option:

- What is known about its effectiveness?
- Is it relevant to New Zealand serotypes and New Zealand conditions?
- Is it commercial-ready?
- Is it permitted in New Zealand?
- Is it available or currently in use in New Zealand?

Such a study requires consultation with the egg industry and should also consider peer-reviewed publications of *Salmonella* interventions applied in broiler farms for their applicability to layer farms. It might also include some economic analyses to compare the cost-effectiveness of each option or other relevant economic indicators (e.g. cost per bird, cost per egg).

Instead, this section provides an introduction to several control options that were considered in recent scientific publications, under the two themes of animal management and environmental management. Some of these options do not appear to be commercially ready, or evaluations were restricted to experimental and limited field studies. A review by Trampel *et al*. (2014) provides a useful summary of interventions targeting *S.* Enteritidis but most of the content is applicable to all serotypes (Trampel *et al.*, 2014).

Animal management

Obtaining chicks from *Salmonella*-free breeding farms is one of the first steps to prevent *Salmonella* infection in flocks. Different resistance to *Salmonella* has also been observed

between poultry breeding lines, and breeding stocks of poultry more resistant to this pathogen will increase control (Doyle and Erickson, 2012).

Hens may be vaccinated against *Salmonella* with live-attenuated vaccines or vaccines prepared from killed bacteria (bacterins). Vaccination has been credited with a marked (70%) reduction of *Salmonella* in eggs in the United Kingdom (UK) since 1998.[35] The advantages of live-attenuated vaccines is the ease of administration (orally) and induction of immunity through activating the antibody and cell-mediated immune responses (Desin et al., 2013). Disadvantages are that serology can no longer be used to distinguish vaccinated animals from pathogen-infected animals, and the risk of reversion to the virulent state. Vaccinations based on *Salmonella* strains with defined mutations are being developed to enable differentiation between vaccinated and infected birds (Doyle and Erickson, 2012). Inactivated/killed vaccines remove the risk of reversion to a virulent state but need to be administered by injection. An Australian study has found that inoculating chickens with live and attenuated vaccines provided the best protection against multiple *Salmonella* serotypes, but their approach required off-label vaccine usage and they did record some adverse effects (Groves, 2011).

International research has reported that vaccination of chickens, along with other control measures as part of a comprehensive *Salmonella* control program is an important strategy in lowering the prevalence of *Salmonella* in poultry flocks, leading to reduction in food-borne *Salmonella* human infections. In the UK, a temporal relationship between *Salmonella* vaccination programs in layer and broilers, and the reduction in human disease, is compelling and suggests that these programs have made a major contribution to improving public health (O'Brien, 2013). However, it is generally agreed that vaccination is most effective when used as part of a *Salmonella* control strategy that includes other biosecurity and sanitising procedures, and does not alone confer protection against *Salmonella* infections amongst flocks. For example, a USA study using environmental drag swabs in small-medium sized farms found the presence of infected rodents and the absence of an *S*. Enteritidis vaccination program were both important risk factors with respect to the prevalence of *Salmonella* (Wallner-Pendleton *et al.*, 2014), i.e. rodent control was just as important as a vaccination programme.

Vaccination of layer hens against *Salmonella* is optional for New Zealand layer farm operators. The RMP template guidelines recommend use of Megan®Vac1, which is a live-attenuated vaccine administered in three doses to day old chicks in the hatchery, then at 2-6 weeks and 13-16 weeks of age.[36] It is a vaccine that is recommended as an aid in the reduction of *S*. Typhimurium, *S*. Enteritidis and *S.* Heidelberg colonisation of the internal organs of young growing chickens and as an aid in the reduction of *S*. Enteritidis colonisation of the crop and digestive tract, including the ceca.

In a USA study of broiler farms, vaccination of breeding hens with Megan®Vac1 followed by a bacterin of the serotypes Berta and Kentucky significantly lowered the prevalence of *Salmonella* in the breeder birds and in their chicks compared with farms that did not vaccinate the breeders (Dorea et al., 2010). Vaccinated hens had a lower prevalence of *Salmonella* in the ceca (38% versus 64% for unvaccinated hens; $p<0.001$) and in the reproductive tracts (14% versus 52%; $p<0.001$). Downstream effects were also noted, with lower *Salmonella* prevalences in environmental samples and broilers where vaccinated breeders were used.

A review of *Salmonella* vaccines has recently been published (Desin *et al.*, 2013). The review found the effectiveness of vaccination to have varying success under experimental and field

[35] http://avianforum2013.merial.com/Documents/lectures/15-Main_challenges_in_poultry_meat_egg_safety-PWigley.pdf accessed 20 December 2015
[36] http://foodsafety.govt.nz/elibrary/industry/template-eggs/Guidelines_Completing-These_Attached.pdf (accessed 14 December 2015).

studies, but most reviewed studies focused on *S.* Enteritidis. Further studies provide examples of variable results:

- A vaccine containing killed *S.* Typhimurium, *S.* Enteritidis and *S.* Kentucky increased the immunity of the hens and their progeny against these particular serotypes but did not decrease the incidence of *Salmonella* in environmental samples taken from the housing (Berghaus *et al.*, 2011).
- An evaluation of the effectiveness of four vaccination programmes used by the UK poultry industry reported that vaccination did not influence the proportion of hens shedding *S*. Enteritidis and *S*. Typhimurium but significantly decreased the incidence of *S*. Enteritidis and *S*. Typhimurium present on eggshells compared to non-vaccinated hens (Arnold *et al.*, 2014a).

Various prebiotics and probiotics introduced through the diet have also been investigated for their effectiveness against *Salmonella*.[37] *Salmonella* infection and shedding may be reduced by administering organic acids through feed and water, particularly medium-chain fatty acids such as caproic, caprylic and capric acid, and by feeding coarse ground meal (Berge and Wierup, 2012; Doyle and Erickson, 2012). Other feed additives include mannan-oligosaccharides (carbohydrates that can bind with *Salmonella* and prevent intestinal attachment), plant-derived antimicrobials (e.g. trans-cinnamaldehyde, carvacrol) and egg proteins (Berge and Wierup, 2012; Darre *et al.*, 2014). Prebiotic feed additives may be most effective when used during periods of higher stress (Doyle and Erickson, 2012). For example, a prebiotic mix containing inulin and oligosaccharides was shown to be effective at reducing the occurrence of *S.* Enteritidis in the cloacae of layer chicks during the first week after they were inoculated with this pathogen (Murate *et al.*, 2015).

The administration of probiotics involves birds ingesting one or more strains of non-pathogenic bacteria to encourage a competitive environment in the gut that helps to create poor conditions for pathogenic bacteria (Doyle and Erickson, 2012). This might involve administering lactic acid bacteria or giving a mixture of bacteria typically found in the gastrointestinal tract of chickens to newly hatched chicks to accelerate establishment of a healthy intestinal microflora (competitive exclusion) (Berge and Wierup, 2012). Competitive exclusion cultures are commercially available but often not approved for use because they consist of mixtures of unidentified bacteria (compared with probiotics that contain one or more well-characterised strains of bacteria) (Berge and Wierup, 2012). Administering probiotics and prebiotics simultaneously (synbiotics) can be more effective than either treatment alone (Doyle and Erickson, 2012) but this has not been consistently reported (Murate *et al.*, 2015).

Bacteriophages have been investigated for efficacy in reducing *Salmonella* colonisation in poultry (Doyle and Erickson, 2012). In one study, regularly dosing growing chicks with bacteriophage reduced horizontal transmission from *S.* Enteritidis-infected chicks to uninfected chicks, plus reduced intestinal *S.* Enteritidis colonisation and environmental contamination (Lim *et al.*, 2012b). Another study did not find bacteriophage therapy to be effective in adult birds challenged with *S.* Enteritidis, but the phage was given to the hens 24 hours prior to the *S.* Enteritidis, so the opportunity for direct contact between the phage and the *Salmonella* cells was limited (Borie *et al.*, 2011).

Bacteriocins are non-toxic antimicrobial peptides secreted by bacteria that have a different mode of attachment and action compared to antibiotics, in that they do not require receptors to bind to bacterial cells and act by damaging the bacterial membrane (Doyle and Erickson,

[37] An older review by Vandeplas *et al.* (2010) provides a useful consolidation of information on dietary modifications and feed additives (Vandeplas *et al*., 2010). This review was not cited in the 2011 Risk Profile.

2012). Nisin is the most widely used bacteriocin in food production, but no recent studies were located where bacteriocins were used to control *Salmonella* on layer farms.

Hens naturally moult after their first cycle of eggs. Moulting can be induced by feed restriction and this is used as a way to stimulate multiple egg-laying cycles, however moulting has been shown to increase *Salmonella* shedding (Berge and Wierup, 2012). New Zealand egg producers are not permitted to induce moulting in their hens.[38] If hens are kept through one or more natural laying cycles, *Salmonella* interventions might target the moulting period. Alternatively, shell eggs produced after moulting may be directed to pasteurised or heat-treated products (Doyle and Erickson, 2012).

Environmental management

Keeping food and water *Salmonella*-free involves ensuring feed is heat treated to reduce or eliminate *Salmonella* and water is from a potable source or sanitised (e.g. chlorinated). The storage and delivery systems must also be routinely sanitised, particularly since these can become a point source for infection of the flock.

Controlling *Salmonella* contamination in the wider environment of the layer house is more challenging. Rodents and insects (e.g. darkling beetles, flies) can transfer *Salmonella* into and throughout poultry housing. Biosecurity interventions including footwear changing, hand sanitising, fly and rodent traps and insecticide spraying are important, however trials of barn-raised poultry in the USA showed that these measures alone were not enough to prevent *Salmonella* from entering the barns over an eight-week period (Dale et al., 2015).

The effectiveness of cleaning and disinfection programmes is variable and is affected by the protocols followed and the condition of the facilities (Doyle and Erickson, 2012). A Canadian study revealed that *S*. Enteritidis was still recoverable from swabs taken from hatcheries after cleaning (S. Enteritidis was isolated from 106/1057 samples, from 85 testing rounds) (Brooks *et al.*, 2012). Trampel *et al*. (2014) have recommended sanitising procedures for layer houses, which include dry cleaning, wet cleaning and fumigation (Trampel *et al.*, 2014). This review highlighted the importance of effective dry cleaning for ensuring subsequent wet cleaning was effective, but did not evaluate different physical or chemical sanitising options. Alternative sanitisers to halogen-based chemicals are being evaluated, e.g. slightly acidic electrolysed water was effective against *Salmonella* naturally present on the equipment and surfaces of a hen house (Hao *et al.*, 2013).

5.2.2 Post-lay controls

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

Like Section 5.2.1, this section does not present a full review and evaluation of all post-lay controls for *Salmonella*.

As discussed in Section 2.5.4, storage of eggs under refrigeration is important for controlling growth of *Salmonella* that may have contaminated the egg contents. Slow cooling after laying is recommended to prevent contraction of the egg contents, but refrigeration should begin promptly after laying.[39]

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[38] http://eggfarmers.org.nz/egg-farming-in-nz/the-code-of-welfare-2012 (accessed 2 December 2015).
[39] In response to public debate over egg refrigeration at retail in Australia, FSANZ has maintained the position that retailers are not required to refrigerate whole, un-cracked eggs. The reasons include the short time eggs spend at retail relative to the entire shelf-life, and that salmonellosis outbreaks linked to eggs are usually caused by consumption of uncooked or lightly-cooked foods. See

Eggs for sale in New Zealand must be visibly clean. Egg washing/sanitising is optional in New Zealand. Egg washing/sanitising may reduce *Salmonella* contamination on the surface of eggs but may also increase the likelihood of shell penetration, although experimental studies are inconsistent on the latter point (see sections 2.4.2 and below).

There is some evidence to suggest that survival and penetration of *Salmonella* is affected by egg washing, but the results from studies are inconsistent. Egg washing, which is used to remove any faeces and reduce the microbial load on the egg surface, is common practice in Australia, the USA and Japan. In Europe, washing of Grade A table eggs is not allowed on the basis that washing increases the likelihood of spoilage and moisture loss from the egg contents (Zhang *et al.*, 2011). In New Zealand, the procedures for operators who choose to undertake egg washing are included in the RMP template for eggs.

When performed correctly, commercial egg washing procedures reduce the microbial load on the eggshell surface which limits the chances of bacteria penetrating the shell as well as limiting cross-contamination of other food items during handling in the kitchen (Gole *et al.*, 2014c). However, there are some detrimental outcomes associated with egg washing such as damage to the physical barriers of the egg, particularly damage to the cuticle (Chousalkar *et al.*, 2010; Samiullah *et al.*, 2013). When performed incorrectly, factors such as the use of wash water with a temperature lower than that of the egg can cause a pressure differential which can draw *Salmonella* from the shell surface into the egg contents (Food Standards Australia New Zealand, 2009).

Alternative chemical sanitisers (e.g. hydrogen peroxide, electrolyzed water) and physical sanitisers (e.g. irradiation, ultraviolet light, whole-egg pasteurisation) are being investigated for their efficacy against *Salmonella* spp., and their effect on the quality and integrity of the egg (Galis *et al.*, 2013; Howard *et al.*, 2012).

The New Zealand RMP for eggs permits application of mineral oil to shell eggs. Laboratory studies indicate potential for *Salmonella* control through alternative shell coatings, e.g. coating eggs with chitosan and natural antimicrobials reduced the concentration of *S.* Enteritidis inoculated onto egg shells (Jin *et al.*, 2013). The treatment also reduced the weight loss of shell eggs during storage.

Section 2.4.4 discusses pasteurisation and cooking as controls for processed eggs.

Pasteurisation regimes that involve multiple hurdles are being investigated but do not appear to be commercially-ready at this stage (Espina *et al.*, 2014; Monfort *et al.*, 2012).

5.2.3 Egg storage

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

The New Zealand RMP for eggs sets out temperature controls for eggs after collection.[40] Eggs should be transported to the grading room, or stored in cool rooms operated at or below 15°C within two hours of collection. Eggs stored in cool rooms on the farm are required to be transported in clean enclosed vehicles at or below 15°C to an off-farm grading facility.

Shelf life options for storage of “A grade” shell eggs and “commercial eggs” include:

- 21 days where the storage/holding temperature may exceed 15ºC

http://www.foodstandards.gov.au/media/Pages/Statement-on-egg-food-safety-.aspx (accessed 15 March 2016).

[40] http://www.foodsafety.govt.nz/elibrary/industry/template-eggs/template.pdf (accessed 7 December 2015).

- 35 days if stored or held at 15°C or less, or
- Other combination to be specified, and justified, by the producer.

Cracked eggs must be stored for a maximum of 14 days at ≤6°C before being pasteurised or treated equivalently.

These shelf-life options apply from the date of lay and the packhouse storage temperature.

It is suggested that the actual practice in New Zealand differs substantially (Ministry for Primary Industries (MPI), 2015) and there is currently no requirement for retailers in New Zealand to store eggs under temperature controlled conditions. Information on best before dates gathered informally during preparation of this Risk Profile suggests that eggs retailed at room temperature in New Zealand supermarkets are carrying the longer, 35 day, best before date.

There has been increased interest from the New Zealand egg industry in determining whether the storage time could be extended for eggs produced in New Zealand, particularly with respect to the increasing export markets. The evidence presented in this document demonstrates that, if present on the shell of eggs, *Salmonella* may survive, but will not multiply. Thus the main issue for egg storage time in New Zealand is the potential for *Salmonella* to penetrate the shell and grow in the egg contents.

A recently published review undertaken by MPI used data up to 2011 to examine the ability of *Salmonella* on whole eggs to penetrate the shell and grow during storage and determine whether current New Zealand handling and storage requirements are justifiable (Ministry for Primary Industries (MPI), 2015). This report posed the question:

> *"If New Zealand were to adopt similar storage recommendations to Australia, allowing a 35 day storage period irrespective of storage temperature, would the number of foodborne illnesses attributed to eggs increase?"*

MPI concluded that "it would appear prudent to maintain the current requirements for handling and storage of eggs". This was based on:

(i) The prevalence of *Salmonella* on the outside of New Zealand eggs;

(ii) The potential for serotypes present in New Zealand to penetrate the egg shell, where survival or growth is possible; and

(iii) Calculations of YMT and *Salmonella* growth that show the potential for *Salmonella* to grow in the egg contents to a concentration that could cause human illness, within the existing New Zealand shelf-life conditions.

Regarding point (i), the supporting evidence was the 2007 New Zealand survey demonstrated that *Salmonella* was be present on the shells of 1.8% of whole eggs at retail. No further data since that survey have become available.

Regarding point (ii), more recent scientific evidence presented in this document continues to support the view that the *Salmonella* serotypes present in New Zealand can potentially penetrate the egg shell. Data published since the MPI review and presented in Section 2.4.2 shows that egg shell penetration was measurable at 21 days at warm temperatures (20-37°C) and at 14 days at 6°C (*S.* Infantis only). Although data for shorter time periods are not available, the high percentage of eggs with detectable shell penetration in these experiments showed there is potential for *Salmonella* to penetrate the shells of whole eggs well within the current shelf-life specifications for New Zealand. However, egg shell penetration experiments

using *Salmonella* serotypes and at temperatures and times appropriate to New Zealand conditions would provide better supporting information for decision making.[41]

Regarding point (iii), the calculations were based on the YMT model of Whiting *et al*. (2000) and the "Rosso" model for *Salmonella* growth in chicken meat, as described in the AECL risk assessment (Thomas *et al.*, 2006).

Some experimental evidence suggests that the YMT model overestimates the time before *Salmonella* growth might occur in 20% of eggs, and therefore underestimates the risk to human health (Food Standards Australia New Zealand, 2009; Ministry for Primary Industries (MPI), 2015). In particular, one study found that growth of one *S*. Enteritidis and two *S*. Typhimurium isolates grew in more than 25% of inoculated eggs after eight days at 20°C (Cogan *et al.*, 2004).[42] This is at or below the lower 95% confidence interval of the YMT model, as generated by (Thomas *et al.*, 2006) derived from data given by (Whiting *et al.*, 2000) . Both of the *S*. Typhimurium isolates tested gave this result, and this is of interest given the importance of this serotype in New Zealand. For the other *S*. Enteritidis isolates tested in the experiments of Cogan *et* al. (2004), the percentage of eggs exhibiting growth was lower, indicating the variability of growth potential for different isolates.

Once growth of *Salmonella* begins in eggs, it is rapid. In the study above, from a starting inoculum of 2-3 cells per egg, the *Salmonella* concentration reached >6 $\log_{10}$ CFU/ml after eight days at 20°C (Cogan *et al.*, 2004).

Based on these published models and assuming an internal contamination of one *S.* Typhimurium cell, MPI estimated a YMT of 28.1 days at 15°C (i.e. exponential growth of *Salmonella* is possible in 20% of eggs at this time and temperature), and that the bacteria will multiply by 1 log (i.e. from one to ten cells) within the following 12 hours (Ministry for Primary Industries (MPI), 2015). At 20°C, the YMT was estimated as 17.2 days and a 1 log multiplication could occur in just under five hours.

No scientific literature published since 2011 has been found that would further inform the YMT model.

The published data since 2011 support the *Salmonella* in whole egg growth model developed for the AECL (Thomas *et al.*, 2006). The maximum growth rates calculated by McAuley *et al.* (2015) for the serotypes Sofia and Typhimurium at three temperatures in liquid whole egg appear to fit within the 95% confidence intervals of the model. While the data for this model are from experiments with whole egg and may overestimate growth rates, studies with intact eggs showed that a growth rate of approximately 1 $\log_{10}$ CFU/day is possible (Cogan *et al.*, 2004). The work of Lublin *et al.* (2015), where *S.* Infantis was inoculated into the yolk of whole eggs, suggests that this serotype grows much slower in egg yolk. Therefore the growth model utilised by MPI would overestimate the risk from this serotype (i.e. fail safe). Further work with serotypes relevant to New Zealand would improve both the YMT and growth model.

The potential for *Salmonella* to grow and reach high numbers inside eggs depends on a combination of factors affecting penetration, breakdown of intrinsic internal defences, and growth rates. In the absence of short term experimental data, it should be assumed that penetration of egg shells can occur at any time after lay, if shell contamination is present. YMT calculations are based on data for the number of days after which >20% of eggs will permit rapid growth, and hence growth may occur earlier in a lower proportion of eggs. Once initiated, growth is very rapid, and more recent data have supported the earlier growth model. In our

[41] A time-series experiment monitoring the proportion of whole eggs penetrated by *Salmonella* at set points during the storage time would be most informative.
[42] In this study, growth was defined as >6 $\log_{10}$ CFU/ml egg.

opinion, the scientific literature since 2011 reviewed for this Risk Profile support the conclusion of the MPI review.

In Australia, the Code of Practice states that eggs shall be stored and transported within a system that avoids excessive temperature fluctuations at all stages until they reach the consumer.[43] The recommended temperature for egg storage is 15°C±3°C (or below) at the farm, during transport and at the retail outlet, in conditions which avoid surface condensation or contamination. However, as in New Zealand, intact shell eggs are usually not stored under temperature controlled conditions at retail.[44] Currently in Australia, egg products are stored up to six weeks (42 days) after packing, even at temperatures where *Salmonella* would grow well if it were present inside the egg.

The above discussion has not included specific controls for *S.* Enteritidis, which is not currently a concern in New Zealand. *S.* Enteritidis is recognised internationally (not in New Zealand) as the serotype most often associated with egg-borne salmonellosis because of its ability to contaminate the interior of intact eggs during their formation within the hen. Recent international studies have shown that survival and growth of *S*. Enteritidis in the contents of shell eggs is influenced by the temperature and time profile for egg storage (EFSA Panel on Biological Hazards (BIOHAZ), 2014; Gross *et al.*, 2015; Health Canada, 2013; Pouillot *et al.*, 2014). In addition to supporting growth of *S.* Enteritidis already in the egg contents, higher temperatures and longer storage times also favour loss of membrane integrity, which makes it easier for *S.* Enteritidis to cross the vitelline membrane and reach the yolk (Pouillot *et al.*, 2014). Data from studies show that refrigeration reduces the risk of internally contaminated table eggs becoming a vehicle for *S.* Enteritidis (EFSA Panel on Biological Hazards (BIOHAZ), 2014; Galis *et al.*, 2013; Health Canada, 2013; Martelli and Davies, 2012). Prompt refrigeration to temperatures capable of restricting microbial growth has been recommended as an approach to reducing the likelihood that contaminated eggs will transmit *S.* Enteritidis to humans (Gast and Holt, 2000). Another study also recommended that the temperature values for shell eggs storage should not exceed 20°C, but ideally be kept below 10°C (Martelli and Davies, 2012)

5.2.4 Microbiological monitoring for *Salmonella*

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

Microbiological surveys of whole eggs in New Zealand and overseas indicate that *Salmonella* are infrequently isolated from the shells or contents (Section 2.5.1, Appendix A.4). Many of these surveys tested eggs from multiple farms and packhouses. Moreover, recent experiments using eggs inoculated with *S*. Enteritidis showed that recovering very low concentrations (<10^3 CFU/egg) is difficult (Webb *et al.*, 2014).

Salmonella contamination of eggs may occur on the layer farm (birds and environment) or the grading/packing facilities (equipment and environment). Contamination of eggs may be sporadic (due to a contamination event, e.g. a batch of contaminated feed) or chronic (e.g. reoccurring due to *Salmonella* surviving cleaning regimes, reintroduction from resident wildlife). Given a low prevalence in eggs, environmental surveys are more likely to detect sources of *Salmonella,* particularly chronic sources, along the egg producing chain. Data on

[43] Code of Practice for shell egg, production, grading, packing and distribution https://www.aecl.org/resources/codes-of-practice/ (accessed 9 December 2015).
[44] Recently Australian Woolworths supermarkets have begun to refrigerate eggs (http://ausfoodnews.com.au/2016/01/13/the-great-egg-debate-fsanz-addresses-concerns.html, accessed 2 February 2016).

the potential prevalence of *Salmonella* in New Zealand eggs might be more usefully obtained through surveys at the layer farms and grading/packing facilities.

This section will consider approaches to surveying layer farms for *Salmonella*, and whether data generated from such surveys can be used to predict the prevalence of *Salmonella* on or in eggs. Additional information on *Salmonella* surveying programmes in breeding flocks and pullets has been included in Appendix C.

Salmonella monitoring programmes have been implemented for layer flocks in the EU and the USA and their requirements are set out in TABLE 7. The key driver for these programmes has been human illness as a result of eggs contaminated with *S.* Enteritidis (EU and USA) and *S.* Typhimurium (EU). The approach in both regions differs, but both use faecal sampling (directly or via boot swabs/socks) with sampling of dust as an alternative.

TABLE 7: *Salmonella* testing schemes for layer flocks in the EU and USA

REGION	SAMPLING FREQUENCY	SAMPLES TAKEN	POOLING OF SAMPLES BEFORE ANALYSIS?	REFERENCE
EU	By operator: At least every 15 weeks. First sampling at the flock-age of 24 +/– 2 weeks. By competent authority: In one flock per year per holding comprising at least 1000 birds[1]	*Cage flocks*: 2x150 g naturally pooled faeces from all belts or scrapers in the house after running the manure removal system. *Step cage houses without scrapers or belts*: 2x150 g of mixed fresh faeces from 60 different places beneath the cages in the dropping pits.	Yes: 2x150 g faeces pooled, 25 g subsample tested	(European Commission, 2011)
		Barn or free-range flocks: 2 pairs of boot swabs or socks[2]	Yes: 2 pairs pooled	
		Alternative sampling options: Replacement of one faecal sample or one pair of boot swabs by a dust sample of either (i) 100 g dust collected from multiple places throughout the house from surfaces with a visible presence of dust or (ii) moistened fabric swabs of multiple surfaces throughout the house (at least 900 cm^2 surface area in total using one or more swabs).	Yes: Dust swabs pooled	
USA	Once for each group of laying hens in a poultry house aged 40-45 weeks	*All layer systems*: Drag swabs of faeces using sterile 12 ply gauze pads (10x10 cm): Number and location of swabs depends on layout.	No	(United States Food and Drug Administration, 2009, 2011)

[1] Additional sampling required where *Salmonella* contamination has been detected or is suspected.

[2] Boot swabs must be absorptive to soak up moisture and the surface of the boot swab must be moistened using appropriate diluents. The samples must be taken while walking through the house using a route that produces representative samples for all parts of the house or the respective sector. This shall include littered and slatted areas provided that slats are safe to walk on. All separate pens within a house must be included in the sampling. On completion of the sampling in the chosen sector, boot swabs must be removed carefully so as not to dislodge adherent material.

It is notable that neither sampling scheme includes samples from individual birds. Two European studies found that taking cloacal swabs from randomly selected hens was less useful for detecting *Salmonella* than taking faecal samples (Schulz *et al.*, 2011; Van Hoorebeke *et al.*, 2010). The researchers concluded that this was probably because of the relatively low within-flock *Salmonella* prevalence of shedding hens (<7%), even in a *Salmonella*-contaminated environment. Testing the caeca or reproductive organs of birds can only be done post-mortem, so is only useful when a flock has been culled at the end of lay, at which time the eggs produced by the birds have already entered the food chain. Testing the reproductive organs is useful for indicating the prevalence of infected but non-shedding birds. Arnold *et al*. (2010) found that environmental sampling was more effective for detecting *S.* Enteritidis contamination than testing the caecal contents and ovaries/oviduct of spent hens (Arnold *et al.*, 2010a).

It is also notable that both schemes focus on faecal sampling, although the method of faecal sampling differs (faeces vs. drag swabs of faeces). Boot swabs/socks will pick up other matter in barn or free-range environments (e.g. dust, food, plant detritus such as straw, grass or sawdust) and can be the preferred method for floor systems (Watanabe *et al.*, 2012). There is general agreement in the literature that pooled faecal samples are effective for detecting *Salmonella*, particularly if the samples include fresh (moist) caecally-discharged faeces (Gast *et al.*, 2015).[45] Testing pooled faecal samples is more sensitive for detecting in-flock infection than testing individual faecal samples, despite the potential for dilution of positive samples by *Salmonella*-free samples (Arnold *et al.*, 2011).

However, studies have found the combination of dust and faecal sampling to be more successful in detecting *Salmonella* (Arnold et al., 2011; Schulz et al., 2011; Watanabe et al., 2012). For example, Schulz *et al*. (2011) found the flock prevalence of *Salmonella* was lower when the results from dust sampling (25 g pooled dust from 20 locations) and faecal sampling (5 pooled 250 g samples) were considered separately (Schulz *et al.*, 2011).

The number of dust and faecal samples taken also influences the results. Arnold *et al*. (2010) compared three environmental testing methods for detecting *S.* Enteritidis and found that the most sensitive testing regime was their in-house method because this incorporated the most samples (Arnold *et al.*, 2010a). The testing regimes compared were:

- The EU method shown in TABLE 7 (two tests: 2 x 150 g faecal samples, pooled, plus 1 x 250 ml dust sample);
- An EU baseline survey method (seven tests: 5 x 200–300 g composite faecal samples or five pairs of boot swabs, plus 2 x 250 ml dust samples); and
- Their in-house method (20 tests: 10 x 25 g composite faecal samples, plus 10 x 15 g dust samples, all collected using a gauze swab moistened with buffered peptone water).

While the EU method had the highest sensitivity on a per sample bases, the in-house method incorporated a larger number of samples and this increased the likelihood of detecting non-uniform environmental contamination. The researchers also found dust sampling to be more sensitive than faecal sampling because *Salmonella* are able to survive better than other Enterobacteriaceae in dry conditions.

[45] Faeces are discharged from the caeca a few times a day and is characteristically brown, soft and moist with a strong odour, *c.f.* normal droppings which are firm, green to brown in colour with some white urate (Gast *et al.*, 2015).

Collecting dust samples is not always possible in caged flocks.[46] In overseas studies, dust samples were replaced by swabs from different locations (Schulz *et al.*, 2011).

The EU and USA methods also differ in their timing and frequency of sampling (TABLE 7). In the EU, operators are required to sample every 15 weeks once the flock is 24+/-2 weeks old. The USA requirements are for a single survey when the flock is aged 40-45 weeks. The USA approach was based on a study that found higher numbers of *Salmonella*-positive environmental samples when laying hens were aged 40-45 weeks. In Denmark, sampling is required every two weeks from the flock age of 20 weeks, but this increased frequency of sampling allows egg producers to market their eggs as *Salmonella*-free (provided *Salmonella* were not detected) (DTU Food, 2015), T. Hald, Technical University of Denmark, pers. comm.).

It is important to recognise that, irrespective of the frequency of sampling, environmental testing will only provide a snapshot of the *Salmonella*-status of a flock at the time of sampling. There are a number of factors that influence the detection of *Salmonella*-infected flocks, including flock housing, the manure handling system, flock size, stage of lay and vaccination (EFSA Panel on Biological Hazards (BIOHAZ), 2014; Galis *et al.*, 2013). Birds infected with *Salmonella* may naturally clear the infection between sampling periods.

Understanding the relationship (if any) between the results of environmental surveys of layer housing and the prevalence of *Salmonella* on eggs in New Zealand requires additional work. Initially, it requires a full understanding of the relationship between inter-flock prevalence (as indicated by environmental sampling), intra-flock prevalence (as indicated by individual bird sampling) and prevalence on eggs at the point of lay, based on New Zealand conditions.

We recommend that a separate study be commissioned to address this question. Such a study might involve:

- Reviewing scientific studies where environmental and egg samples were analysed for *Salmonella* contamination under both experimental conditions (controlled introduction of *Salmonella*) and commercial conditions (testing flocks of unknown or known *Salmonella*-status). Under what conditions was there a relationship between a *Salmonella*-positive flock and *Salmonella*-positive eggs? Are these conditions relevant to the New Zealand situation?[47]
- Reviewing risk assessments where environmental or flock prevalence data were used to calculate egg prevalence. Can these approaches be applied to the New Zealand situation?
- Collection of New Zealand-specific data where necessary.
- An investigation based on mathematical modelling to provide probability predictions for the prevalence of *Salmonella*-positive eggs in New Zealand, given a prevalence of *Salmonella*-positive flocks.

Some overseas field studies have reported compelling evidence to suggest a correlation between the number of positive environmental samples and the proportion of eggs positive in a flock, suggesting that prevalence of infection and on-farm hygiene are directly related to the number of contaminated eggs produced (Arnold *et al.*, 2014b; Dewaele *et al.*, 2012b; Gole *et al.*, 2014d).

[46] See http://eggfarmers.org.nz/news/media-statements/where-do-our-eggs-come-from for a useful video of New Zealand layer farms (accessed 1 December 2015).
[47] E.g. studies in other countries often focus on *S.* Enteritidis.

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APPENDIX A: HAZARD AND FOOD

A.1 *SALMONELLA*: TYPING METHODS

The serotyping and phage typing methods were described in the 2011 Risk Profile, which also briefly introduced the molecular typing methods of PFGE and MLVA. Further information on these and other molecular techniques is included here. Because molecular methods are much faster than serotyping (and eliminate the need for holding stocks of antibodies), effort has been directed towards developing a molecular-based method that can rapidly and reliably identify the serotype of an isolate (Ranieri *et al.*, 2013).

PFGE continues to be the standard molecular method for *Salmonella* typing. The method involves using a restriction enzyme that targets a very small and specific sequence that appears mulitple times within bacterial DNA, and cuts the DNA at this point. This results in the DNA being cut into different sized fragments that are then visualised on a gel. As reported in the 2011 Risk Profile, PFGE is used for salmonellosis outbreak/cluster investigations in New Zealand. ESR routinely uses one enzyme for PFGE, but additional enzymes may be used if further differentiation between strains is required. PFGE is an internationally-recognised technique and results can be compared through the database PulseNet.[48]

The limited discriminatory power of PFGE for *S*. Enteritidis strains and clusters has been reported and has prompted researchers to evaluate and develop other molecular typing methods, namely MLVA and multilocus sequencing typing (MLST).

MLVA is an increasingly popular method of molecularly subtyping bacterial foodborne pathogens. In comparison to other subtyping methods, MLVA is a relatively new technology that is made possible by recent advances in whole genome sequencing (WGS). The MLVA method has been investigated as an alternative to phage typing due to its superior speed of analysis and ability to differentiate closely related strains (Cuttell *et al.*, 2015). The technique targets a small number of loci within a bacterial genome that exhibit a broad range of variable number tandem repeats (VNTR). VNTR loci are initially selected by interrogating whole genome sequences for short tandem nucleotide repeats using a specialized software package. MLVA is used in New Zealand if PFGE does not result in sufficient discriminatory power (Muriel Dufour, ESR Enteric Reference Laboratory, personal communication 10 Sept. 2015). In Australia, a national MLVA typing network has been established since 2006 where notifications of human salmonellosis caused by *S*. Typhimurium are characterised by phage and MLVA typing.

MLST relies on sequencing genes associated with enzymes. The genes of interest include multiple housekeeping genes that are present in all strains within in a species, family or other defined group of bacteria because they are essential for maintaining cellular function. These genes evolve very slowly and providing a reliable measure of genetic relationships between bacterial isolates (Urwin and Maiden, 2003). Each housekeeping gene or locus may consist of multiple alleles which can be differentiated based on gene sequence. Each different allele, whether it varies in sequence (one or multiple nucleotides) or in size, is assigned an arbitrary number. The sequence type of each isolate is determined based on the combination of numbers representing each of the alleles present in that particular isolate. Sequence types that contain many of the same alleles can then be further grouped into sequence type complexes or clonal complexes or clonal groups (Hauser *et al.*, 2013).

[48] http://www.pulsenetinternational.org/ (accessed 16 November 2015).

MLST is reasonably laborious to perform but is highly reproducible and easily standardised for comparison between different laboratories (Karama and Gyles, 2010). MLST has been suggested to possibly be more accurate for predicting pathogenicity and host preferences (Achtman *et al.*, 2012). The discriminatory ability of MLST for the typing of *Salmonella* has been reported to be better than that of serotyping and/or PFGE (Kotetishvili *et al.*, 2002). MLST is not routinely used for typing *Salmonella* isolates from human cases of salmonellosis in New Zealand.

The rapid development of sequencing technologies worldwide has seen a surge of research applying WGS to many foodborne pathogens, including *Salmonella*. It has been reported that WGS accurately identifies many *Salmonella* serotypes, as well as distinguishes between strains to an extent comparable to other typing methods such as PFGE ((Ranieri *et al.*, 2013; Wattiau *et al.*, 2011; Zhang *et al.*, 2015). WGS promises to deliver high-resolution genomic epidemiology as the ultimate method for bacterial typing but many challenges, including the development of standard protocols and analyses, need to be addressed before WGS-based surveillance systems can be implemented (den Bakker *et al.*, 2014; Sabat *et al.*, 2013). Public Health England have begun to routinely conduct WGS of all presumptive *Salmonella enterica* received by the reference laboratory. It is hypothesised that the increased information provided by WGS can be used to detect outbreaks and provide insight into the epidemiology of known outbreaks (Ashton *et al.*, 2015). The technique has been used to investigate outbreaks of salmonellosis in Europe (Ashton *et al.*, 2015).

An extension of WGS involves identifying whole-genome derived single nucleotide polymorphisms (SNPs), and this has been found to improve cluster resolution and has proven useful in epidemiologic investigations involving *Salmonella* infection attributed to eggs (Ashton *et al.*, 2015; den Bakker *et al.*, 2014; Fabre *et al.*, 2012; Hawkey *et al.*, 2013; Inns *et al.*, 2015; Lienau *et al.*, 2011). It is now being developed as a routine method for surveillance because it provides greater insight into the epidemiology of identified outbreaks (Ashton *et al.*, 2015). Several SNP typing schemes have been developed for analysis of *S*. Enteritidis. Through the discovery of SNPs in more variable regions of the genome it has been possible to discriminate separate lineages of this clonal organism, particularly those involved in outbreak investigations (Bakker *et al.*, 2011; den Bakker *et al.*, 2014; Lienau *et al.*, 2011).

A.2 ANTIMICROBIAL RESISTANCE OF NEW ZEALAND *SALMONELLA* ISOLATES

ESR tests the antimicrobial resistance of approximately 20% of all human and non-human *Salmonella* isolates received for typing, along with all *S*. Typhimurium phage types that are internationally recognised as being multiresistant.[49]

Resistance to each of the 12 antimicrobials tested and multiresistance to three or more of these is shown in TABLE 8 for human isolates, and TABLE 9 for non-human isolates (isolates from animal or environmental samples), for the years 2010 to 2014.

The percentage of human or non-human *Salmonella* isolates that demonstrate antimicrobial resistance is low each year (usually 5% or less). Between 2010 and 2014, the percentage of isolates from humans that was resistant to three or more antimicrobials was between 3.1 and 9.9 per year. For non-human isolates this range was 0.6-2.9%. When the human and non-human isolates are combined, the percentages that were fully susceptible to all 12 antimicrobials each year were high, although this appears to be decreasing: 92.0% (2010), 90.3% (2011), 88.2% (2012), 86.9% (2013) and 85.5% (2014).

[49] Data are available from the annual reports of antimicrobial susceptibility among *Salmonella*, produced by ESR and available at: http://www.surv.esr.cri.nz/antimicrobial/salmonella.php (accessed 30 November 2015).

TABLE 8: Antimicrobial resistance of a sample of New Zealand *Salmonella* isolates from humans, 2010-2014[1]

ANTIMICROBIAL	PERCENT OF ISOLATES RESISTANT EACH YEAR (n=number tested)				
	2010 (n=235)	2011 (n=222)	2012 (n=230)	2013 (n=235)	2014 (n=205)
Ampicillin	7.7	10.4	8.3	10.1	9.8
Cephalothin	0.6	0.5	0.9	2.0	2.4
Chloramphenicol	0.8	3.2	2.6	3.1	3.9
Ciprofloxacin	0.2	0.5	0.0	0.0	1.0
Co-amoxiclav	0.4	0.5	0.4	0.8	1.5
Co-trimoxazole	0.6	1.8	5.2	2.3	2.4
Gentamicin	0.6	1.4	1.7	1.2	0.5
Nalidixic acid	3.9	8.6	9.6	6.6	8.3
Streptomycin	2.7	8.1	7.0	7.0	7.3
Sulphonamides	3.7	8.1	9.1	8.6	6.8
Tetracycline	3.7	11.3	9.1	11.3	9.3
Trimethoprim	0.6	1.8	5.2	2.3	2.4
Multiresistant to ≥3 antimicrobials[2]	3.1	9.9	9.1	9.0	7.8

[1] Data are from the annual reports of antimicrobial susceptibility among *Salmonella*, produced by ESR and available at: http://www.surv.esr.cri.nz/antimicrobial/salmonella.php (accessed 2 September 2015).

[2] For all years, for estimates of multidrug resistance, ciprofloxacin and nalidixic acid resistance, and co-trimoxazole and trimethoprim resistance, was counted as one resistance.

TABLE 9: Antimicrobial resistance of a sample of New Zealand *Salmonella* isolates from animal and environmental samples, 2010-2014[1]

ANTIMICROBIAL	PERCENT OF ISOLATES RESISTANT EACH YEAR (n=number tested)				
	2010 (n=252)	2011 (n=284)	2012 (n=203)	2013 (n=182)	2014 (n=140)
Ampicillin	0.8	1.8	2.5	0.0	2.9
Cephalothin	0.0	1.1	0.5	0.0	0.7
Chloramphenicol	0.4	0.0	0.5	0.6	1.4
Ciprofloxacin	0.0	0.0	0.0	0.0	0.0
Co-amoxiclav	0.4	0.0	0.0	0.0	0.0
Co-trimoxazole	0.4	0.4	0.0	0.0	0.7
Gentamicin	0.0	1.1	0.0	0.0	1.4
Nalidixic acid	0.0	1.1	0.5	1.1	2.9
Streptomycin	1.6	2.8	2.0	2.2	2.1
Sulphonamides	2.0	2.5	1.5	3.3	1.4
Tetracycline	1.6	2.1	1.5	0.6	4.3
Trimethoprim	0.4	0.4	0.0	0.0	0.7
Multiresistant to ≥3 antimicrobials[1]	1.2	1.8	1.0	0.6	2.9

[1] For all years, for estimates of multidrug resistance, ciprofloxacin and nalidixic acid resistance, and co-trimoxazole and trimethoprim resistance, was counted as one resistance.

A general trend throughout the period 2010-2014 was for *Salmonella* isolated from humans to be significantly ($p<0.05$) more resistant to ampicillin, nalidixic acid, sulphonamides and tetracycline than *Salmonella* isolated from other sources. *Salmonella* isolates from humans were also significantly ($p<0.05$) more multiresistant than *Salmonella* isolated from other sources. An analysis of trends in resistance, published for the years 2008 to 2013, reported significant ($p<0.05$) increases in resistance to ampicillin and tetracycline.

For the years from 2010 to 2014, *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.

The antimicrobial susceptibility of all isolates belonging to internationally recognised multiresistant *S*. Typhimurium clones is tested. These clones include *S*. Typhimurium phage types DT104, U302, DT12, DT120 and DT193. Another multiresistant *Salmonella* clone has been recognised recently – *Salmonella enterica* serovar 4,[5],12:i:-. *S*. *enterica* serovar 4,[5],12:i:- is considered a monophasic variant of *S*. Typhimurium, and multiresistant isolates are typically resistant to ampicillin, streptomycin, sulphonamides and tetracycline. From 2010, the antimicrobial susceptibility of all *S. enterica* serovar 4,[5],12:I:- isolates has been tested. TABLE 10 summarises details of the prevalence and multiresistance status of these *Salmonella* types during the period 2010-2014.

TABLE 10: Prevalence of known multiresistant *Salmonella* types in New Zealand (isolates from humans and animals), 2010-2014

TYPE	NUMBER OF ISOLATES OF TYPE TESTED MULTIRESISTANT/NUMBER OF ISOLATES OF TYPE (NUMBER FOR WHICH OVERSEAS TRAVEL IDENTIFIED)[1]				
	2010	2011	2012	2013	2014
DT104	1/1	2/2 (1)	-	-	-
U302	0/2	1/1	1/1 (1)	-	1/1
DT120	1/1 (1)	3/3 (2)	2/3 (1)	-	1/5 (1)
DT193	0/1	0/4 (2)	0/22	1/14	3/27 (1)
DT12	-	-	-	0/1	-
4,[5],12:i:-	13/21 (7)	22/22 (11)	38/38 (12)	24/35 (19)	24/27 (23)
Comment	All human isolates	One poultry isolate (4,[5],12:i:-), remainder human isolates	All human isolates	Four DT193 isolates were from animal sources, remainder human isolates	Ten DT193 isolates were from animal sources; remainder human isolates

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

Fluoroquinolone (ciprofloxacin)-susceptible strains of *Salmonella* that are resistant to the older-generation quinolone nalidixic acid may be associated with clinical failure or delayed response when fluoroquinolones are used to treat extra-intestinal *Salmonella* infections. In 2010 and 2011, one ciprofloxacin resistant isolate was identified each year, with an additional 18 nalidixic acid resistant isolates each year.

In 2012, the additional ciprofloxacin interpretive standards specifically for typhoidal and extraintestinal non-typhoidal *Salmonella* infections were introduced. While none of the non-typhoidal *Salmonella* tested in 2012 were categorised as ciprofloxacin resistant using the interpretive standards applied to intestinal infections, three isolates from human sources (0.7% of all non-typhoidal *Salmonella* tested and 1.3% of human isolates) would be categorised as resistant using the standards applied to extraintestinal infections. In 2013, the interpretive

standards for ciprofloxacin were changed again to a uniform set of breakpoints applicable to all *Salmonella*. Previously the interpretive standards for typhoidal *Salmonella* and extraintestinal non-typhoidal *Salmonella* infections differed from those for other *Salmonella*. With the application of the new standards, none of the non-typhoidal *Salmonella* tested in 2013 were categorised as ciprofloxacin resistant and two were resistant in 2014.

A.3 OVERSEAS DATA: *SALMONELLA* ON LAYER FARMS

A.3.1 Australia

Surveys have been conducted in two Australian states (New South Wales and Queensland) to provide baseline data relevant to establishing, validating and verifying measures to control *Salmonella* at the farm level (Cuttell *et al.*, 2015; NSW Food Authority, 2013a). The results from these have been summarised in TABLE 11.

TABLE 11: Results from surveys for *Salmonella* on egg farms in New South Wales and Queensland[1]

VARIABLE	NEW SOUTH WALES	QUEENSLAND
Year of survey	2010/11	2014
Number of farms surveyed	49	21
Number of farms positive for *Salmonella*	22 (45%)	12 (57%)
Number of farms positive for *S.* Typhimurium	10 (20%)	3/21 (14%)
Number of farms positive for *S.* Enteritidis	0	0
Bulk stored feed: Number of samples positive for *Salmonella*	3/27 (11%)	0/21
Drinking water supply: Number of samples positive for *Salmonella*	0/20	0/21
Feed at point of consumption: Number of samples positive for *Salmonella*	17/101 (17%)	Not tested
Drinking water at point of consumption: Number of samples positive for *Salmonella*	3/46 (6%)	Not tested
Boot/cage swabs: Number of samples positive for *Salmonella*	26/99 (26%)	20/53 (38%)
Boot/cage swabs: Number of samples positive for *S.* Typhimurium	9/99 (10%)	3/53 (6%)
Faeces: Number of samples positive for *Salmonella*	15/90 (17%)	15/53 (28%)
Faeces: Number of samples positive for *S.* Typhimurium	8/90 (9%)	4/53 (8%)

[1] From (Cuttell *et al.*, 2015; NSW Food Authority, 2013a).

In total, seventeen different serotypes were isolated across the *Salmonella*-positive egg farms in the New South Wales survey and fifteen were isolated in the Queensland survey. *S.* Typhimurium was isolated most often in both surveys and accounted for 30% (39/130) of all the *Salmonella*-positive samples from New South Wales and 20% (7/35) of all the *Salmonella*-positive samples from Queensland. *S.* Infantis was the second most-often serotype isolated in the New South Wales study (25/130, 19%), and the third most often serotype isolated in the Queensland study (4/35, 11%, equal to *S.* Agona).

S. Typhimurium isolates were analysed by MLVA in both studies. Seven different MLVA types were identified in the New South Wales study and five MLVA types were identified in the Queensland study. None were the same between studies. Two MLVA types in the New South

Wales study were common among notified human cases (MLVA 3-9-7-13- 523 and MLVA 3-9-7-15-523).

Between January 2011 and October 2015 FSANZ issued five recalls for eggs and three of these were for potential contamination of *Salmonella*.[50] The other two recalls were for potential microbial contamination due to cracked eggs.

A.3.2 European Union

The most recent report available for the EU (EFSA and ECDC, 2015) summarises data on *Salmonella* prevalence among laying hen flocks for the 2013 year. The ultimate EU target for laying hen flocks is a maximum of 2% of adult flocks positive for *S.* Enteritidis and/or *S.* Typhimurium. Reporting of monophasic *S.* Typhimurium si included within the *S.* Typhimurium total.

Data for 2013 were reported by 28 member states and three non-member states. Overall, the EU-level prevalence of adult laying hen flocks positive with *Salmonella* spp. was 2.6% (3.2% in 2012). The EU-level prevalence of adult laying hen flocks positive with *S.* Enteritidis and/or *S.* Typhimurium was 1% (1.3% in 2012). A decreasing trend has been evident since 2008. Five member states and two non-member states reported no flocks positive with *S*. Enteritidis and/or *S*. Typhimurium. *S.* Enteritidis was more commonly isolated compared with *S.* Typhimurium (0.8% Enteritidis, 0.2% Typhimurium).

Two additional studies have been reported for the UK:

- A model, based on surveillance data on *Salmonella* occurrence in flocks of laying hens and assuming sampling of only one flock per holding, estimated that 18% (95% CrI 12-25%) of egg-laying holdings in the UK were infected with *Salmonella* (Arnold *et al.*, 2010b).
- Estimated within-flock prevalences ranged from <1% to 67% among *S.* Enteritidis infected flocks from 21 laying houses in the UK (Arnold *et al.*, 2010a). These estimates were based on *Salmonella* testing of the ovaries/oviduct and caeca dissected from individual chickens taken from these infected flocks. Prevalences were higher in non-caged flocks, however, there was no significant difference in the concentration of positive samples between farms with high or low prevalences.

A.3.3 Japan

A nationwide survey of *Salmonella* spp. in dust from layer farms identified 48/203 (24%) farms that were positive for *Salmonella* spp. (Iwabuchi *et al.*, 2010). From 380 isolates, the serotypes Infantis, Agona and Mbandaka were the most prevalent (each 10-11% of isolates) and most isolates from these serotypes were also resistant to one or more antibiotics.

A.4 OVERSEAS DATA: *SALMONELLA* ON OR IN EGGS

The 2011 Risk Profile 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%. *Salmonella* was more likely to be detected on the outside of the egg or when the whole egg (shell and contents) were analysed together.

Results from surveys published from 2011 have been summarised in TABLE 12. All are from Australia, Korea and Japan. The surveys continue to show low *Salmonella* prevalence on and

[50] http://www.foodstandards.gov.au/industry/foodrecalls/recalls/Pages/default.aspx (accessed 18 November 2015). Older recall information kindly supplied by FSANZ.

in eggs (<1%). An exception is the 2005-2010 Japanese survey of egg contents (13% positive) but it must be noted that the egg was purchased from retail as pooled, liquid eggs.

A 2012 survey of 75 samples of raw and undercooked egg products from food businesses in Victoria, Australia, did not detect *Salmonella*.[51] The samples included mayonnaise, aioli, tiramisu, hollandaise, and tartare sauce.

[51] http://www.foodstandards.gov.au/science/surveillance/Pages/microbiologicalsurve5556.aspx (accessed 15 March 2016).

TABLE 12: Prevalence of *Salmonella* spp. on and in eggs sampled from countries similar to New Zealand (published in the scientific literature since 2011)

COUNTRY	SURVEY PERIOD	SAMPLE SOURCE	TARGET MICROORGANISM	PORTION ANALYSED[1]	NUMBER OF EGGS SAMPLED	NUMBER OF POOLED SAMPLES (NUMBER OF EGGS POOLED)	NUMBER OF POSITIVE POOLED SAMPLES (%)	CALCULATED PREVALENCE[2]	SEROTYPES ISOLATED	REFERENCE
Australia	NR	Farms	*Salmonella* spp.	Unbroken	1560	260 (6)	NR[3]	0.45%[3]	Infantis, 4,12:d	(Chousalkar and Roberts, 2012)
				Contents	1560	260 (6)	0	NA	NA	
Australia	NR	Farms	*Salmonella* spp.	Unbroken	1860	310 (6)	14 (4.5%)	0.8%	Infantis, 4,12:d	(Gole *et al.*, 2013)
				Shell	1860	310 (6)	0	NA	NA	
				Contents	1860	310 (6)	0	NA	NA	
Korea	NR	Markets, retail	*Salmonella* spp.	Shell	60	NA	0	NA	NA	(Park *et al.*, 2015)
Japan	2005-2010	Retail[4]	*Salmonella* spp.	Contents[5]	30	30[4]	4	13%	Enteritidis, Montevideo, Braenderup	(Murakami *et al.*, 2013)
Japan	2007-2008	Retail	*Salmonella* spp.	Shell	20300	2030 (10)	5	0.2%	Enteritidis, Derby, Livingstone, Cerro	(Sasaki *et al.*, 2010)
				Contents	20300	2030 (10)	0	NA	NA	
Japan	2008-2009	Farms	Senftenberg	Contents	281470	9383 (30)[5]	0	NA	NA	(Shirota *et al.*, 2012)
Japan	2010-2011	Retail	Enteritidis	Contents	105033	5400 (20)[5]	3 (0.06%)	0.0029%	NA	(Esaki *et al.*, 2013)

NR, not reported; NA, not applicable.

[1] Unbroken = whole unbroken eggs analysed; shell = broken or crushed shell analysed without contents; contents = contents of the egg analysed without the shell; whole = egg broken and shell and contents analysed together.

[2] Assuming only one egg out of the pooled sample was contaminated.

[3] The authors' reported that 5/26 flocks were positive and that seven *Salmonella* isolates were serotyped (6 *S*. Infantis), but the number of pooled samples that were positive was not reported. Ten pooled samples (i.e. 60 eggs) were sampled from each flock. Assuming that the seven isolates were identified from seven different pooled samples, and that only one egg was positive in a positive pooled sample, the prevalence is 7/1560, or 0.45%.

[4] Purchased as liquid eggs.

[5] Some pooled samples had less eggs. Esaki *et al*. (2013) reported that some were discarded because they were cracked.

[6] 95% confidence interval 0.0025-0.0032.

APPENDIX B: SALMONELLOSIS OVERSEAS

B.1 INCIDENCE

International comparisons of salmonellosis and the proportions identified as Enteritidis and non-Enteritidis must be made cautiously since laboratory testing protocols and reporting practices often differ.

The 2011 Risk Profile presented incidence data for salmonellosis for Australia, European and North American Countries, mainly for the years 2008-2009. These data showed New Zealand's rate of salmonellosis to be higher than most other developed countries. Australia's salmonellosis rate was noticeably higher than New Zealand's. The most recent incidence data available are given in TABLE 13, with New Zealand data provided for comparative purposes. These data show that New Zealand's salmonellosis rate remains elevated above those of the EU (as a whole), USA and Canada, but Australia's salmonellosis rate continues to be the highest of this group.

Since 2008 there has been a steady decrease in human salmonellosis cases in the EU, particularly cases of *S*. Enteritidis infection. Country-specific trends decreased in the majority of reporting countries between 2008-2012. This suggests a positive public health impact of implementation of various EU-level prevention and control measures, which includes the implementation of *Salmonella* control programmes in the poultry industry since 2008, as well as improved hygiene and education of consumers and food-workers. Nevertheless, salmonellosis is the second most commonly reported enteric infection in humans in the EU (ECDC, 2015).

In Australia, the rate of salmonellosis has increased significantly over the last 10 years which is causing concern for health and food authorities. *S*. Typhimurium notifications in 2011 increased by 50% compared with the five-year mean (2006-2010). *S*. Enteritidis notifications have also seen a large percentage (54%) increase compared with the five-year mean, but the majority of cases in Australia infected by this serotype are associated with overseas travel (The OzFoodNet Working Group, 2015).

The importance of overseas travel has also been demonstrated by a Canadian study, where an analysis of salmonellosis cases caused by *S*. Enteritidis reported between 2003 and 2009 found seasonal patterns associated with phage types. PT8, PT13 and PT13a showed a summer peak and were associated with domestically-acquired infection, and PT1, PT4 and PT6a showed a winter peak and were more likely to be associated with cases who had travelled (Nesbitt *et al.*, 2012). Overall, the reported rate of infection with *S*. Enteritidis increased over the time period analysed, with the increase being attributed to domestically-acquired infection.

TABLE 13: Rates of reported salmonellosis in Australia, the European Union and North America (most recent data available), compared with New Zealand[1]

COUNTRY	YEAR	INCIDENCE (PER 100,000 POPULATION)[1]	CHANGE IN INCIDENCE COMPARED WITH 2009 (2009 rate)[1]	SEROTYPED ISOLATES[2]				
				NUMBER WITH SEROTYPE ASSIGNED	% Enteritidis	% Typhimurium	% Infantis	% other serotypes
New Zealand	2012	24.5	↓ (26.2)	1044	12	44	5	39
	2013	25.6		1141	12	42	6	40
	2014	21.2		958	12	41	6	41
Australia	2012	49.5	↑ (43.8)	NR[3]				
	2013	55.3						
	2014	69.7						
EU/EEA[4]	2012	22.1	↓ (24.0)	82183	41.2	22.2	2.4	34.2
	2013	20.4		73627	39.5	20.2	3.0	37.3
Canada	2012	20.1	↓ (18.1)	6979	30	12	3	55
	2013	17.8		NR				
USA[6]	2012	16.4	↑ (15.0)	7411	16.7	12.4	2.1	68.8
	2013	15.2		6520	19.0	14.1	NR	66.9
	2014[7]	15.5		6565	21.3	12.3	3.6	62.8

[1] References:
New Zealand: (Horn *et al.*, 2015) and Section 3.3.2 of this report.
Australia: (NNDSS, 2015)
EU/EEA: (ECDC, 2015; EFSA, 2007; EFSA and ECDC, 2015; EFSA Panel on Biological Hazards (BIOHAZ), 2014)
Canada: (NESP, 2014)
USA: (Crim *et al.*, 2015; Crim *et al.*, 2014; USCDC, 2011, 2014)

[2] NR, not reported; ↓ rate decreased since 2009; ↑ rate increased since 2009.

[3] Data not available for these years. In 2011, serotype information was available for 98.6% of the 12,271 notified cases (numerator not specified or calculable) (The OzFoodNet Working Group, 2015). Based on the 12,271 cases, 48% were Typhimurium and 7% were Enteritidis.

[4] EEA, European Economic Area. Incidence calculated from data from 25 EU Member States (26 Member States in 2009), serotypes calculated from data from 25 member states and two non-member states. Cases of Enteritidis and Typhimurium are decreasing.

[6] Data is for the 10 sentinel states monitored by FoodNet, not the whole of the USA, and is for laboratory confirmed cases only. Data for 2013 and 2014 are preliminary.

B.2 COMMUNITY LEVEL ESTIMATES

The number of notified salmonellosis cases only represents a proportion of total cases, as not all cases will come into contact with public health agencies. The 2011 Risk Profile reported the followed community level estimates for salmonellosis:

- Australia: Rate of 262 (95% CrI: 150-624) per 100,000 people, based on notified cases from 2000 through 2004 (Hall *et al.*, 2008).
- USA: Rate of 366 domestically-acquired cases per 100,000 people, based on surveillance data from 2000 to 2008 and the 2006 USA population of 299 million(Scallan *et al.*, 2011).
- England and Wales: 73,193 domestically-acquired foodborne cases, based on data from 1996-2000 (Gillespie *et al.*, 2005).

The global burden of (circa 2006) was estimated at 93.8 million cases, with 155,000 deaths (Majowicz *et al.*, 2010). The incidence was estimated as 1,140 per 100,000 person-years.

New estimates for the annual number of salmonellosis cases and rates of infection have been published:

- Australia: Rate of salmonellosis circa 2000 was 150 (90% CrI 80-270) per 100,000 population, and for circa 2010 was 185 (90% CrI 100-335) (Kirk *et al.*, 2014). These are lower than the estimates by Hall *et al*. (2008).
- France: Rate of 307 (90% CrI 173-611) cases per 100,000 (Van Cauteren *et al.*, 2015)

B.3 OUTBREAKS

This section does not provide a full overview of recent salmonellosis outbreaks linked to eggs that have been reported in other countries similar to New Zealand. Many of the egg-associated outbreaks in North American and European countries are caused by *S.* Enteritidis, which is of less importance in New Zealand. Instead, this section includes information on recent outbreaks reported in Australia, outbreaks of non-Enteritidis salmonellosis linked to eggs that have been reported in other countries and the results from relevant reviews of public health surveillance data.

B.3.1. Outbreaks in Australia associated with eggs

There has been an increase in salmonellosis outbreaks associated with egg consumption in Australia (The OzFoodNet Working Group, 2015). *S*. Typhimurium has been the most frequent causative serotype, despite *S.* Infantis being the predominant serotype in the Australian egg industry (Samiullah *et al.*, 2013). The reason for this increase is not clear but a common element of many of these outbreaks is the consumption of raw or undercooked eggs, particularly in desserts and sauces, and the use of dirty and/or cracked eggs. This is evidenced by the outbreaks listed in TABLE 14, from 2012 and 2013. Additional outbreaks have been reported during 2014 and 2015. For example, foods containing raw egg condiments were implicated in an outbreak of salmonellosis in March 2014 that involved over 200 cases, and fried ice cream prepared with an egg-based batter was the suspected cause of a 2015 outbreak that affected over 100 people.[52]

[52] http://www.theage.com.au/victoria/egg-warning-after-salmonella-outbreak-20140303-33z36.html and http://www.abc.net.au/news/2015-01-08/more-than-110-people-ill-after-suspected-salmonella-outbreak/6006958 (accessed 1 December 2015).

TABLE 14: Outbreaks of salmonellosis associated with eggs, Australia, 2012-2013[1]

DATE	SEROTYPE, MLVA TYPE[2]	NO. ILL	SETTING	IMPLICATED FOOD
Nov.13	Typhimurium PT9	3	Private residence	Suspected pasta carbonara containing raw eggs
Nov.13	Typhimurium PT135	27	Hospital	Suspected undercooked eggs
Nov.13	Typhimurium PT170/108 MLVA 03-09-07-14-524	20	Restaurant	Chocolate mousse containing raw egg
Nov.13	Typhimurium PT16 MLVA 03-13-10-12-524	350	Commercial caterer	Potato salad with raw egg mayonnaise
Oct.13	Typhimurium PT9 MLVA 03-24-12-10-523	11	Restaurant	Raw egg aioli
Oct.13	Typhimurium PT170 MLVA 03-10-07-14-523	49	Bakery	Mayonnaise made with raw egg
Sep.13	Typhimurium PT9 MLVA 03-24-12-10-523	15	Restaurant	Raw egg aioli
Jul.13	Typhimurium PT135a	12	Restaurant	Eggs
Jul.13	Typhimurium PT16 MLVA 03-13-10-11-524	30	Café	Eggs benedict
Jun.13	Typhimurium PT9	2	Private residence	Raw egg mayonnaise
Jun.13	Typhimurium PT9 MLVA 03-23-23-11-523	17	Private residence	Béarnaise sauce
May13	Typhimurium PT44	36	Restaurant	Tartare sauce/aioli (raw eggs)
May13	Typhimurium PT 170/108 MLVA 03-09-07-13-523	161	Restaurant	Potato salad containing raw egg mayonnaise
Mar.13	Typhimurium PT44	22	Restaurant	Scrambled eggs
Mar.13	Typhimurium PT9 MLVA 03-15-06-11-550	9	Restaurant	Eggs
Mar.13	Typhimurium MLVA 03-17-09-12-523	4	Private residence	Raw egg smoothies
Feb.13	Typhimurium PT9	4	Private residence	Caesar salad dressing containing raw egg
Feb.13	Typhimurium MLVA 03-09-07/08-14-523	7	Restaurant	Fried ice cream with raw egg
Jan.13	Typhimurium PT135a	10	Private residence	Tiramisu containing raw eggs
Jan.13	Typhimurium PT135	3	Correctional facility	Raw egg drink
Dec.12	Typhimurium PT170	3	Private residence	Raw egg drink
Nov.12	Typhimurium 135a	5	Private residence	Chocolate mousse made with raw eggs (suspected)
Sep.12	Typhimurium PT9	11	Restaurant	Fried ice cream made using raw eggs
Aug.12	Typhimurium PT16 MLVA 03-13-11-11-524	3	Restaurant	Chicken Caesar salad with raw egg dressing
Aug.12	Typhimurium PT170 MLVA 03-09-08-14-523	14	Commercial caterer	Raw egg mayonnaise

DATE	SEROTYPE, MLVA TYPE[2]	NO. ILL	SETTING	IMPLICATED FOOD
Jul.12	Typhimurium PT135a	7	Private residence	Chocolate mousse containing raw eggs
Jun.12	Typhimurium PT170 MLVA 03-09-07-12-523	3	Restaurant	Ice cream containing raw egg
May12	Typhimurium MVLA 03-09-09-12-523	12	Restaurant	Fried ice cream (undercooked or made with raw eggs)
Apr.12	Typhimurium PT4	4	Private residence	Raw egg smoothies
Apr.12	Typhimurium PT135	44	Other	Suspected raw egg mayonnaise and/or tartare sauce
Apr.12	Typhimurium PT170 MLVA 03-09-09-12-523	5	Restaurant	Fried ice cream (undercooked or made with raw eggs)
Apr.12	Typhimurium PT135a	20	Restaurant	Eggs Benedict
Mar.12	Typhimurium MVLA 03-12-13-09-524	5	Restaurant	Fried ice cream, suspected
Mar.12	Typhimurium PT170 MLVA 03-09-07-12-523	22	Restaurant	Raw egg white emulsions
Mar.12	Typhimurium PT44 MLVA 03-10-08-09-523	11	Takeaway	Vietnamese egg rolls with raw egg butter
Mar.12	Typhimurium PT170 MLVA 03-09-09-12-523	18	Restaurant	Raw egg products, suspected (Bombe Alaska)
Feb.12	Typhimurium PT141	8	Takeaway	Egg-based sauces (consumed with seafood)
Feb.12	Typhimurium PT170 MLVA 03-09-09-12-523	9	Restaurant	Fried ice cream (potentially undercooked eggs)
Feb.12	Typhimurium PT170 MLVA 03-09-08-13-524	10	Restaurant	Raw egg mayonnaise
Feb.12	Typhimurium PT170 MLVA 03-09-07-13-523	20	Restaurant	Raw egg mayonnaise
Jan.12	Typhimurium PT170 MLVA 03-09-07-12-523	14	Restaurant	Fried ice cream (potentially undercooked eggs)
Jan.12	Typhimurium PT170 MLVA 03-09-07-13-523	5	Restaurant	Eggs and omelettes (undercooked)
Jan.12	Typhimurium MVLA 03-13-10-10-524	4	Private residence	Chocolate cake with raw egg meringue

[1] Data compiled from the OzFoodNet Quarterly Reports, available from http://www.ozfoodnet.gov.au/internet/ozfoodnet/publishing.nsf/content/reports-1 (accessed 1 December 2015) and (Moffatt *et al.*, 2012)

[2] PT, phage type (definitive type). MLVA, multiple-locus variable-number tandem repeat analysis profile.

The most recent annual public health surveillance report available, for 2011, reported 33 outbreaks of salmonellosis during that year that were attributed to a single food category, of which 30 were due to *S.* Typhimurium infection (The OzFoodNet Working Group, 2015). Of the 30 outbreaks of *S.* Typhimurium infection, 29 outbreaks (involving 487 cases) were associated with egg-based dishes, most of which included raw or undercooked egg as an ingredient. Analytical epidemiological evidence or microbiological evidence was available to implicate the egg-based dishes in 12/29 outbreaks. Further descriptive information in the report revealed that in two outbreaks the traceback investigations resulted in the outbreak strain being isolated from layer farms supplying the eggs to food service outlets.

A recent paper has reported on a series of seven outbreaks of *S.* Typhimurium DT135a in Tasmania in 2005, 2007 and 2008 that were linked to raw egg-containing foods where the eggs were sourced from the same farm (Hawkey *et al.*, 2013).[53] Together, these outbreaks involved 193 microbiologically-confirmed cases. The outbreak strain was isolated from the farm in December 2005 and January 2006, which subsequently ceased to operate.

An investigation of a 2008 outbreak of *S*. Typhimurium infection in New South Wales found the cause to be eggs from a single supplier (the outbreak strain was isolated on the farm) and one practice that likely contributed to the outbreak was the practice of 'wet wiping' faeces from soiled eggs (Craig *et al.*, 2013).

B.3.2. Outbreaks of non-Enteritidis salmonellosis linked to eggs and reported in other countries

TABLE 15 lists details of six outbreaks of non-Enteritidis infection linked to eggs. These are only outbreaks that were reported in the scientific literature since this usually provides the best available information on risk factors, evidence for eggs as the vehicle of infection and any failures that led to egg contamination.

These outbreaks demonstrate that eggs can be contaminated with serotypes other than Enteritidis (and Typhimurium), which can lead to outbreaks of salmonellosis. Not all of the reports specify whether the eggs were undercooked or raw. Cross-contamination from the raw eggs may also have contaminated the foods consumed, e.g. contamination via food handlers or surfaces.

One recently reported international outbreak of *S.* Enteritidis infection is worth noting because it clearly demonstrates how a contamination event can impact multiple regions when eggs are distributed widely from a single point of origin. Eggs from a single packing centre in Germany were linked to sporadic or outbreak cases in Austria, France, Germany, Luxembourg and the UK, and a total of 229 cases were confirmed as being infected by the outbreak strain (Inns *et al.*, 2015).

[53] DT135a is a variant of *S.* Typhimurium DT135 that is commonly reported in Australia.

TABLE 15: Overseas outbreaks of *Salmonella* Typhimurium linked to eggs (reported in the scientific literature from 2011)

COUNTRY	YEAR	EGG PREPARATION	SEROTYPE	NUMBER OF CASES	EVIDENCE	POSSIBLE FAILURE(S)	REFERENCE
Japan	2008	Tamagotoji (soft egg dish made with unpasteurised liquid eggs	Braenderup	176	Cohort study	Use of contaminated eggs	(Mizoguchi *et al.*, 2011b)
France	2009	Raw egg (tiramisu)	Non-motile variant of Typhimurium (antigenic formula 4,5,12: –:–)	8	Outbreak strain isolated from tiramisu	On-farm contamination: Outbreak strain isolated from dust and faecal samples on layer farm	(Le Hello *et al.*, 2012)
China	2012	Egg sandwiches	Chester and Enteritidis	56	Case control study, Enteritidis outbreak strain isolated from egg sandwich from another batch	Use of contaminated eggs	(Guo *et al.*, 2015)
Jersey	2013	Raw egg (mayonnaise)	Typhimurium DT8	21	Common food, outbreak strain isolated from another batch of mayonnaise	Use of duck eggs contaminated on-farm	(Ashton *et al.*, 2015)
Multiple: Airline travellers returning from Tanzania	2011	Milk tart or egg dish	Heidelberg	22	Case control study	Not identified.	(Rebolledo *et al.*, 2014)
Ireland	2009-2011	Duck eggs	Typhimurium DT8	35	Common food (Mizoguchi *et al.*, 2011a)	On-farm contamination: Outbreak strain isolated from layer	(Garvey *et al.*, 2013)

						farms linked to human cases	

B.3.3. Reviews of relevant surveillance data in other countries

The following has been reported:

- UK, 52 national outbreaks of salmonellosis, 2000-2011 (Harker et al., 2014): Of 32 outbreaks where a suspected vehicle of infection was reported, eggs were reported in 10 outbreaks, involving 2,873 people. This was ranked second highest in terms of the number of outbreaks, behind salad/leaf vegetables (12 outbreaks, 1,315 cases). The serotypes were Enteritidis (6 outbreaks, including one liquid egg), Typhimurium (2 outbreaks, both duck eggs), Virchow/Infantis (1 outbreak) and Bareilly (1 outbreak).
- USA, 403 foodborne outbreaks of salmonellosis, 1998-2008 (Jackson et al., 2013): Eggs were the most commonly implicated food commodity (112 outbreaks, 28%) and of the 112 egg-associated outbreaks, 65/112 (58%) were caused by *S*. Enteritidis and 42/112 (38%) by S. Heidelberg.
- USA, 1325 outbreaks of *S*. Enteritidis infection, 1973-2009 (Wright et al., 2015): From 636 outbreaks where a single food or contaminated ingredient was implicated, egg-containing foods were most commonly implicated (450 outbreaks, 71%).
- EU/EEA, 2013 (EFSA and ECDC, 2015): Of 314 strong-evidence foodborne outbreaks reported, eggs and egg products were the most frequently identified food vehicles and were implicated in 141/314 (45%) outbreaks. Of these 141 egg-associated outbreaks, 88% (124/141) was caused by *S*. Enteritidis and 1% (2/141) was caused by *S*. Typhimurium. In the EU/EEA, *S*. Typhimurium is more associated with outbreaks involving pig meat and products thereof.

B.4 CASE CONTROL STUDIES INVESTIGATING EGGS AS A RISK FACTOR FOR SALMONELLOSIS

TABLE 16 lists details from two recently-published case control studies investigating consumption of eggs as a risk factor for salmonellosis. The Israeli study (Bassal *et al.* 2014) clearly associated *S.* Infantis infection with consumption of eggs. While the Canadian study (Middleton *et al*. 2013) found no association between *S*. Enteritidis infection and consumption or preparation of eggs, additional questioning of a subset of respondents did reveal that respondents who did not wash their hands following handling of raw eggs almost tripled their odds of infection relative to those who reported washing their hands (odds ratio (OR) 2.8, 95% confidence interval (CI) 1.5–5.4).

A recently published systematic review and meta-analysis of 34 salmonellosis case control studies published between 1989 and 2003, that focussed on sporadic disease, found the risk factors “eggs” and “eggs, S. Enteritidis” were not significant, but “undercooked eggs” and “undercooked eggs, S. Enteritidis” were (Domingues *et al.*, 2012). However, the authors suggested that additional (missing) data from small- or medium-sized case control studies might reduce the effect of eating undercooked eggs as risk factor.

TABLE 16: Case control studies published since 2011 considering eggs as a risk factor for salmonellosis

TIME PERIOD	COUNTRY	SEROTYPE	RISK FACTOR	NUMBER OF PARTICIPANTS		NUMBER REPORTING RISK FACTOR		ODDS RATIO (95% CONFIDENCE INTERVAL) BY:[1]		REFERENCE
				CASES	CONTROLS	CASES	CONTROLS	UNIVARIATE ANALYSIS	MULTIVARIATE ANALYSIS	
2009	Israel	Infantis	Consumption of eggs	186	186	154	138	**1.7 (1.0-2.9)**	**1.9 (1.0-3.5)**	(Bassal *et al.*, 2014)
2011	Canada	Enteritidis (domestically-acquired)	Any egg consumption	199	241	98	129	1.0 (0.7-1.5)	NS	(Middleton *et al.*, 2014)
			Runny eggs			29	24	1.8 (0.99-3.2)		
			Away from home[2]			32	31	1.5 (0.9-2.5)		
			Preparation			40	52	1.1 (0.7-1.8)		
			Preparation eggs or foods with raw eggs[3]			27	23	0.9 (0.5-1.8)		
			Consumption of foods with raw eggs[3]			1	0	0.8 (0.02-∞)		

[1] NS, not significant and values not reported; NR, not reported. ORs and CIs rounded to 1 decimal place. Bolding indicates significant results.
[2] Only asked from participants who reported egg consumption.
[3] Only asked 233 respondents.

B.5 ATTRIBUTION STUDIES

Attribution studies apply expert opinion and/or statistical modelling to human health surveillance data to attribute human illness to sources or vehicles of infection. A recent summary of salmonellosis attribution studies showed that the proportion attributed to layers (eggs) varied between regions (e.g. 6% in New Zealand, 44% in the EU), although the authors strongly cautioned that some of the variability was due to different approaches to making the attribution estimates (Pires *et al.*, 2012).

Some recently published salmonellosis attribution studies have considered eggs:

- South Australia: A source attribution model was used to estimate the contribution of different animal reservoirs to illness due to *Salmonella* between 2000 and 2010, and an estimated 37% (95% CrI 23-53) of sporadic salmonellosis cases were attributed to eggs (Glass *et al.*, 2015). Source-related parameters were included to allow for different handling and consumption practices. Analysis of source-related parameters showed higher risk of illness from contaminated eggs than from contaminated chicken meat, suggesting that consumption and handling practices potentially play a bigger role in illness due to eggs, considering low *Salmonella* prevalence on eggs.
- USA: Based on outbreak analysis, 11.8% of foodborne salmonellosis was estimated to be due to egg consumption, while expert elicitation estimated a higher percentage (21.8%) (Batz *et al.*, 2012). A lower estimate was produced from a modelling study that estimated 6% of sporadic, domestically-acquired salmonellosis cases could be attributed to consumption of egg products (Guo *et al.*, 2011). By comparison, 48% was attributed to chicken, 28% to ground beef and 17% to turkey.
- EU: An attribution model comparing the occurrence of *Salmonella* serotypes in animals and humans predicted that layers were the most important reservoir of human salmonellosis (De Knegt *et al.*, 2014). The model estimated that 42% of cases (7,903,000 cases, 95% credibility interval 4,181,000–14,510,000) were attributable to eggs from laying hens, with the serotype Enteritidis causing 95% of these infections. The report noted possible differences in the epidemiology of *Salmonella*, surveillance focus and eating habits between countries. For example, most cases in Finland and Sweden were travel-related, while in most other countries the main sources were related to the laying hen or pig reservoir. The next most important reservoir was pigs, which was the source of an estimated 31% of cases, and an estimated 13% of cases were attributed to broilers. Similar results were found in an attribution study for sporadic, domestically-acquired salmonellosis in The Netherlands (51% attributed to layers/eggs, followed by pigs at 40%) (Mughini-Gras *et al.*, 2014). In Denmark, an attribution model estimated pork was more important as a source of domestically-acquired foodborne salmonellosis in 2014 than eggs (estimated 15.4% cases attributed to pork vs. estimated 3% attributed to table eggs) (DTU Food, 2015).

B.6 RISK ASSESSMENT AND RISK-RELATED ACTIVITIES OVERSEAS

B.6.1. Australia

As outlined in the 2011 Risk Profile, FSANZ prepared a risk assessment that considered the microbiological and chemical hazards associated with egg consumption in Australia (Food Standards Australia New Zealand, 2009).[54] This included a quantitative risk assessment that considered salmonellosis from eggs, which was based on a model developed at the request

[54] http://www.foodstandards.govt.nz/code/proposals/Pages/proposalp301primaryp3426.aspx. Accessed September 2015.

of the Australian Egg Corporation Ltd (AECL) (Thomas *et al.*, 2006). Information on the FSANZ risk assessment is now available.

The model assumed that eggs were not internally contaminated at the point of lay, and internal contamination arose through salmonellae migrating across the shell and shell membranes. Growth in the egg contents was determined by yolk mean time, which is the time required for *Salmonella* to begin exponential growth as a result of the vitelline membrane degrading. The exposure assessment module of the model incorporated different 'best', 'median' and 'worst' scenarios for egg collection frequency and storage temperatures/times.

The main findings of the model include:

- The length of time until there is potential for rapid growth of *Salmonella* in contaminated eggs depends on the temperature of the egg from point of lay to consumption, i.e. shorter times with increasing storage temperatures.
- For eggs stored under conditions that would permit the growth of *Salmonella* the estimated number of salmonellosis cases was 36 per one million serves of uncooked egg. The estimate was four cases per one million serves of uncooked egg if eggs were stored under conditions that did not permit *Salmonella* growth. Consumption of well-cooked eggs presented little risk of illness.
- A 50% reduction in prevalence of contaminated eggs resulted in a 50% reduction in the risk of illness from raw eggs that have been stored under time and temperature conditions that have allowed *Salmonella* to grow in the yolk.

The report acknowledged a lack of data on exposure of consumers to foods containing uncooked or undercooked eggs or egg products.

B.6.2. Europe

The 2011 Risk Profile cited a quantitative risk assessment published by EFSA in 2010 that considered *S.* Enteritidis in shell eggs in Europe (EFSA, 2010). EFSA used this model to investigate changes to the predicted number of contaminated eggs with a reduction of flock prevalence (EFSA Panel on Biological Hazards (BIOHAZ), 2010).

In 2014, EFSA published a quantitative risk assessment that investigated the possible impact of extending the shelf-life of eggs on the risk to consumers posed by *S.* Enteritidis (EFSA Panel on Biological Hazards (BIOHAZ), 2014). The model was that used in the AECL model but modified to address the European situation (EFSA Panel on Biological Hazards (BIOHAZ), 2014). Different situations of storage times from 7 to a maximum of 70 days were simulated and compared to the actual situation regarding the storage of eggs in the EU ('Best-before date' and 'Sell-by date' is 28 and 21 days after laying, respectively). The results of the model suggested that prolonging the storage time for table eggs increased the number of illnesses per million servings, except when eggs are well-cooked. The magnitude of this increase depended on the additional time of storage that the eggs spent at retail and in households.

B.6.3. Canada

A risk assessment considering the public health outcomes associated with the consumption of shell eggs internally contaminated with *S*. Enteritidis was published by Health Canada in 2011 (DeWinter *et al.*, 2011). The baseline model used a mean prevalence of internally-contaminated eggs of 1.7×10^{-6}, or 1.7 eggs in every million at the point of lay, and considered changes to the concentration of *S.* Enteritidis during storage and meal preparation to estimate exposures. The model estimated a mean of 120 illnesses per year (5% and 95% points of 20 and 280, respectively, meaning that in 5% of nominal years there are less than 20 illnesses and in 5% of nominal years there are more than 280 illnesses). The results from the model also estimated that consumers were 2.7 times more likely to become ill from eggs consumed at a food service or institutional setting than in a home setting, and that poor storage and

handling conditions represented only 0.6% of exposures to resulted in 46% of illnesses. The model was used to simulate various risk management strategies.

The results of the above model were used by Health Canada to produce a guidance document outlining intervention strategies to reduce the risk of *S*. Enteritidis in eggs offered at sale (Health Canada, 2013). The document recommends a number of actions from the layer stage through to the processed egg stage.

B.6.4. Risk ranking studies

Risk ranking approaches can be used to prioritise risk management efforts.

In a recent USA study, the pathogen-food combination *Salmonella*-eggs was ranked 10th in a list of 168 pathogen-food combinations based on the annual disease burden in the USA (cost of illness, QALY loss) (Batz *et al.*, 2011). The burden of disease values and subsequent rankings were based on attributions using outbreak data and expert elicitation.

APPENDIX C: CONTROL MEASURES IN OTHER COUNTRIES

C.1 AUSTRALIA

The Primary Production and Processing Standard 4.2.5 for Eggs and Egg Products was gazetted by FSANZ in May 2011 and has been in force since 26 November 2012. This Standard (which applies to Australia and not New Zealand) was developed in response to the large number of foodborne illness outbreaks suspected of being linked to eggs or egg products, particularly cracked and dirty eggs which have been a key cause of contamination. Full details about the standard including risk assessment reports can be found online.[55]

Overall, the standard will reduce the incidence of illness associated with eggs 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. Implementation of these Schemes is expected to improve egg handling and processing practices resulting in production of safer and cleaner eggs by businesses (NSW Food Authority, 2013b).

A '*Salmonella* Initiative' was introduced by the AECL in September 2014.[56,57] 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. Some of the initial outputs are topic papers. A paper on egg washing has been completed and reviewed (but is not yet available) and other topic papers will include pasteurisation of eggs, *Salmonella* detection and typing, egg storage and transport and *Salmonella* vaccination. Another major output is a 'through-chain *Salmonella* risk identification' report which will outline risks that have been identified through the course of the *Salmonella* Initiative and will include identification of management processes to consider when assessing/managing risks.

[55] http://www.foodstandards.govt.nz/code/proposals/pages/proposalp301primaryp3426.aspx. Accessed September, 2015.
[56] https://www.aecl.org/resources/food-safety/ (accessed December 2015).
[57] The *Salmonella* Initiative – stakeholder update (publication date not available). Provided by MPI, August 2015.

C.2 EUROPE

Several EU regulations exist to prevent *Salmonella*-contaminated eggs from being placed on the market (Inns *et al.*, 2015).[58] 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*.[59]

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 *Gallus gallus*.[60] The Union 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 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.[61]

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*, e.g. may be used for human consumption only if 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 *Gallus gallus* initially through Regulation (EC) No. 1003/2005, with amendments through Regulation (EC) No. 200/2010 and Regulation (EC) No. 213/2009.[62] 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.[63]

TABLE 17 summarises the *Salmonella* sampling scheme for breeding flocks (layers and broilers) and pullets.

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[58] http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=URISERV:f83005 (accessed 7 December 2015).
[59] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:325:0001:0015:EN:PDF (accessed 7 December 2015).
[60] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1168&from=EN (accessed 7 December 2015).
[61] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0517&from=EN (accessed 7 December 2015).
[62] http://ec.europa.eu/food/food/biosafety/salmonella/impl_reg_en.htm (accessed 27 January 2016).
[63] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:061:0001:0009:EN:PDF (accessed 27 January 2016).

TABLE 17: Summary of *Salmonella* sampling scheme for breeding flocks and pullets of *Gallus gallus* in the European Union (Regulation (EC) No. 213/2009)[1]

SAMPLING LOCATION[2]	SAMPLING FREQUENCY	SAMPLES TAKEN
Hatchery	By operator: Every 2 weeks. By competent authority: Every 16 weeks, plus - (i) within 4 weeks following moving to laying phase or laying unit (ii) towards end of laying phase, not earlier than 8 weeks before the end of the production cycle	(i) composite sample of visibly soiled hatcher basket liners; OR (ii) composite sample of one or several moistened fabric swab(s) taken from the bottom of hatcher baskets, or from fluff; OR (iii) broken eggshells taken from separate hatcher baskets (crushed, mixed and sub-sampled to form a 25 g subsample for testing).
Holding	By operator: Every 2 weeks. By competent authority: Three occasions during the production cycle: (i) within 4 weeks following moving to laying phase or laying unit (ii) towards end of laying phase, not earlier than 8 weeks before the end of the production cycle (iii) at any time during the production cycle which is sufficiently distant in time from the above sampling	(i) pooled faeces samples; AND (ii) boot swabs and/or dust samples;

[1] See Regulation (EC) No. 213/2009 for full details, e.g. when sampling can be reduced or must be increased, specifications for the samples required: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:073:0005:0011:EN:PDF (accessed 27 January 2016).

[2] The operator can choose to sample at the hatchery or holding, unless the eggs for hatching are intended for trade within the EU, then sampling must be at the holding.

C.3 USA

The 2011 Risk Profile described the USFDA's Egg Safety Final Rule, which has a focus on *S.* Enteritidis.[64] There have been no changes to this Rule.

64 http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Eggs/ucm170615.htm (accessed 7 December 2015).

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