



# Risk profile: *Salmonella* (non typhoidal) in and on eggs

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

Prepared for the Ministry of Agriculture and Forestry  
under project MRP/10/01, Microbiological Risk Profiles,  
as part of overall contract for scientific services

Client report FW11037

by

Dr Rob Lake  
Dr Sandra Moorhead  
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November 2011

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\*On 30 April 2012, MAF was renamed as the Ministry for Primary Industries (MPI) which was an amalgamation of the New Zealand Food Standards Agency (NZFSA) and MAF. Prior to 2002, NZFSA was the Australia New Zealand Food Authority (ANZFA). MAF was in existence when this Risk Profile was produced in 2011. Unless otherwise stated, this Risk Profile uses the names ANZFA, NZFSA and MAF for documents produced during the existence of these organisations.

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## SUMMARY

The purpose of a Risk Profile is to provide information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. Risk Profiles include elements of a qualitative risk assessment, as well as providing information relevant to risk management.

The food/hazard combination addressed by this Risk Profile is *Salmonella* (non-typhoidal) in and on eggs. This document represents an update of the Risk Profile completed in 2004.

This Risk Profile has been commissioned in order to address the following specific risk management questions:

- What is the public health risk from *Salmonella* in and on eggs consumed in New Zealand?
- Has the risk of salmonellosis from consumption of eggs changed since the 2004 Risk Profile?

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.

*Salmonella* can contaminate eggs internally by two routes;

- trans-ovarian - a vertical transmission; and,
- trans-shell - a horizontal transmission.

Throughout the 1980s and 1990s the incidence of notified cases of salmonellosis fluctuated between 37 and 57 per 100,000 population, with no apparent trend. The rate has declined since a peak of 65 per 100,000 population in 2001, and has been stable in New Zealand since 2005 at 25-35 reported cases per 100,000 population. This rate is close to that in other developed countries, particularly those in Europe, and lower than in Australia.

*Salmonella* Enteritidis is the dominant serovar in layer flocks in Europe and the United States (US) and most *Salmonella* infections attributed to eggs in these countries are also caused by this serovar. As a result, most international research into *Salmonella* in and on eggs has focused on *S. Enteritidis*. Surveys undertaken from New Zealand poultry flocks and eggs have not isolated *S. Enteritidis* and it is therefore not believed to be endemic in New Zealand at this time. Incidents of salmonellosis in New Zealand where eggs are implicated are likely to be caused by external contamination of the eggs, by dirt and/or faecal material which may involve other *Salmonella* serovars. It has been previously reported that there are no consistent differences between *S. Enteritidis* and *S. Typhimurium* in terms of the factors that affect horizontal transmission (Wales and Davies 2011) and consequently, this Risk Profile uses studies on *S. Enteritidis* with the assumption that the information also applies to other serovars of *Salmonella*.

A twelve month survey of eggs in New Zealand undertaken in 2007 did not detect *Salmonella* in the contents, while 1.8% of eggs (9/514 sample units representing 49 different brands, including cage laid, free-range and barn laid systems) were contaminated externally. This value was reported to be higher than values for other developed countries, apart from a 2008 survey in South Australia. Of the New Zealand egg samples that tested positive for *Salmonella*, 4 out of 9 sample units contained “dirty” eggs (obvious contamination of shell with faecal, feather or other organic material). All positive samples were from cage laid eggs, and all isolates were identified as *S. Infantis*.

Periodic spikes of contamination of eggs by *Salmonella*, arising from contaminated inputs such as feed and drinking water, or other environmental sources (rodents, pests etc.), are likely to be the pattern for this food/hazard combination.

A report from a US study commented that even a low rate of contamination can represent a large number of contaminated eggs, given the large numbers produced each year (Braden, 2006). Approximately 1 billion eggs are produced in New Zealand each year

This Risk Profile has been commissioned in order to address the following specific risk management questions:

- What is the public health risk from *Salmonella* in and on eggs consumed in New Zealand?
- Has the risk of salmonellosis from consumption of eggs changed since the 2004 Risk Profile?

The risk of salmonellosis from consumption of eggs does not appear to have changed since the 2004 Risk Profile, based on:

- the static incidence of reported illness, which is almost the same as in 2004;
- data supporting the continued dominance of *S. Typhimurium* types in reported cases, rather than *S. Enteritidis*, which is associated with trans-ovarian transmission.

In domestic and retail food service settings, surface contaminated eggs may contaminate foods when the eggs are cracked for use, and cross contamination may occur during pooling of the eggs. Cross contamination may also occur via hands, utensils and surfaces, or directly to the mouth. Contaminated food may be cooked by methods which will reduce the number of bacteria in the foods but may not entirely eliminate the bacteria.

It is possible that *Salmonella* contaminating the surface of eggs may penetrate into the egg and eventually reach the yolk contents and grow (Zhang et al. 2011). If eggs are refrigerated, then mathematical modelling estimates suggest that this would be a rare occurrence. However, there are only very limited data on the storage conditions and times for eggs by New Zealanders. Egg washing procedures may also create opportunities for shell penetration through cuticle damage, cracking or temperature differentials.

A case-control study in Minnesota found that sporadic cases were significantly more likely to have consumed undercooked eggs, or egg-containing foods during the three days before onset of illness despite no egg-associated outbreaks of salmonellosis being recognised in the region (Hedberg *et al.*, 1993). This situation may apply in New Zealand. Although eggs are unlikely

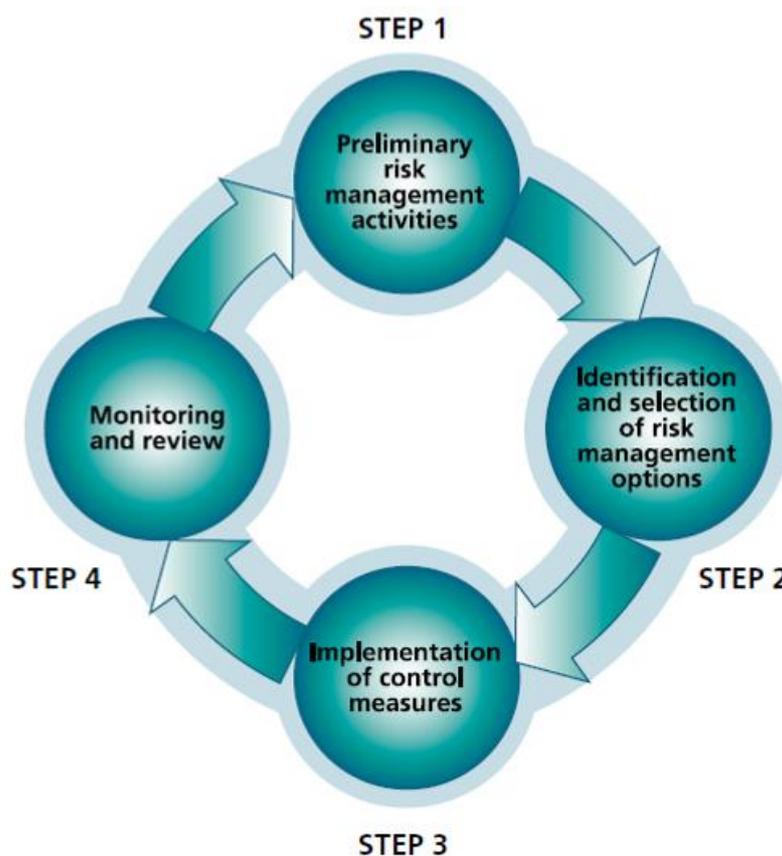
to be a dominant vehicle for transmission of salmonellosis in New Zealand, the estimate that eggs represent the cause of up to 10% of infections appears reasonable (Wilson and Baker, 2009). This would equate to a cost of approximately \$1.5 million based on burden figures reported by Gadiel and Abelson, (2010).

The data gaps identified in this Risk Profile for *Salmonella* in eggs 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.

## 1 STATEMENT OF PURPOSE

The purpose of a Risk Profile is to provide information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. Risk Profiles are part of the Risk Management Framework (RMF)<sup>1</sup> approach taken by the Ministry of Agriculture and Forestry (MAF) Food Safety. The Framework consists of a four step process, as shown in Figure 1.



**Figure 1: The four steps of the Risk Management Framework**

This initial step in the RMF, Preliminary Risk Management Activities, includes a number of tasks:

- Identification of food safety issues
- Risk profiling
- Establishing broad risk management goals
- Deciding on the need for a risk assessment
- If needed, setting risk assessment policy and commissioning of the risk assessment
- Considering the results of the risk assessment
- Ranking and prioritisation of the food safety issue for risk management action.

<sup>1</sup> [http://www.foodsafety.govt.nz/elibrary/industry/RMF\\_full\\_document\\_-\\_11604\\_NZFSA\\_Risk\\_Management\\_Framework\\_3.1.pdf](http://www.foodsafety.govt.nz/elibrary/industry/RMF_full_document_-_11604_NZFSA_Risk_Management_Framework_3.1.pdf)

Risk profiling may be used directly by risk managers to guide identification and selection of risk management options, for example where:

- Rapid action is needed;
- There is sufficient scientific information for action;
- Embarking on a risk assessment is impractical.

## 1.1 Food/hazard Combination and Risk Management Questions

The food/hazard combination addressed by this Risk Profile is *Salmonella* (non-typhoidal) in and on eggs. This document represents an update of the Risk Profile completed in 2004 (Lake *et al.*, 2004a).

This Risk Profile has been commissioned in order to address the following specific risk management questions:

- What is the public health risk from *Salmonella* in and on eggs consumed in New Zealand?
- Has the risk of salmonellosis from consumption of eggs changed since the 2004 Risk Profile?

## 1.2 MAF Risk Management Strategy

In March 2010, MAF (then the New Zealand Food Safety Authority; NZFSA) released their *Salmonella* Risk Management Strategy 2009-2012. The Strategy aims to achieve a 30% reduction in the reported annual incidence of foodborne salmonellosis after five years. The strategy focuses on non-typhoid *Salmonella* and begins with a primary focus on intelligence gathering from a wide range of food sectors.

The objectives of the *Salmonella* risk management strategy are to:

- Quantify the proportion of foodborne cases attributable to:
  - specific foods
  - animal feeds
  - domestically produced versus imported foods
  - multiresistant and virulent *Salmonella* genotypes associated with foods
- Identify sources of *Salmonella* contamination of specific foods and animal feeds
- Determine the relative value of different interventions throughout the food chain in reducing the risk of salmonellosis
- Make prioritised risk management decisions on appropriate *Salmonella* control measures across the food chain, and according to data availability
- Design and implement an effective monitoring and review programme to support strategic goals.

An updated version of the strategy was published in 2010 that covers 2010-2013.<sup>2</sup> This version records NZFSA's progress towards achieving the objectives. Those relevant to this Risk Profile are recorded in Table 1.

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<sup>2</sup> Available at <http://www.foodsafety.govt.nz/industry/general/foodborne-illness/salmonella/strategy.htm>

**Table 1: Egg-specific outputs or results from the NZFSA *Salmonella* Risk Management Strategy**

Work programme	Egg-specific outputs/results	Ref*	See also (in this report)
Systematic review of the epidemiological evidence available within New Zealand of the aetiology of human <i>Salmonella</i> infection (completed)	Eggs are “Likely” to be a “minor cause” (i.e. <10% of foodborne cases).	1	Section 3.3.5.1
Attribution of potentially foodborne enteric diseases: human salmonellosis. Enhanced surveillance including outbreaks (completed)	“There are few confirmed food sources, and of the suspected food sources, some can be discounted on the basis of other evidence. This particularly applies to eggs;”	2	Section 3.3.5.1
Literature review of ability of <i>Salmonella</i> on egg shells to penetrate the shell and grow during storage for up to 35 days (in progress)	A systematic literature review to determine whether <i>Salmonella</i> on the external surface of freshly laid eggs can penetrate the shell and if so, how quickly it will grow at various temperatures over 0 – 35 days after lay when stored at ambient temperatures representative of a NZ summer.		Section 7.3.1.1
Compliance audit of egg processors (completed in 2009)			
Survey of retail eggs for <i>Salmonella</i>	This survey assessed the presence of <i>Salmonella</i> in and on eggs available through retail outlets in Auckland and Christchurch. A total of 514 sample units of eggs were tested over a twelve-month period.	3	

\* References:

1. (Wilson and Baker, 2009)
2. (Adlam *et al.*, 2010; King *et al.*, 2011)
3. (Wilson, 2007)

## 2 HAZARD AND FOOD

The information in this section represents a summary of a microbiological data sheet relevant to this Risk Profile. These data sheets are prepared by ESR for a number of different foodborne pathogens as requested by MAF.<sup>3</sup> Additional information on the hazard and food is included in Appendix 1.

### 2.1 *Salmonella* spp.

This group of bacteria is comprised of two species: *Salmonella enterica*, which is divided into six subspecies (*enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*), and *Salmonella bongori* (Grimont and Weill, 2007). Most pathogenic isolates from humans and other mammals belong to *S. enterica* subspecies *enterica*. Other *S. enterica* subspecies and *S. bongori* are more common in cold blooded animals and the environment, and are of lower pathogenicity to humans and livestock (Brenner *et al.*, 2000; Jay *et al.*, 2003).

*Salmonella* are primarily divided into types using serological identification of somatic (O), flagella (H), and capsular (K) antigens. There are more than 2,500 different *Salmonella* serotypes (also called serovars), and of these over 1,500 have been identified in the *S. enterica* subspecies *enterica* group (Grimont and Weill, 2007).

*S. enterica* subspecies *enterica* serotypes are given serotype names (Jay *et al.*, 2003). The full name and serotype name are normally abbreviated to a shortened form, where the serotype is capitalised and non-italicised, e.g. *Salmonella enterica* subsp. *enterica* serotype Enteritidis becomes *Salmonella* Enteritidis (or *S. Enteritidis*). In older publications this may be represented as a species name i.e. *Salmonella enteritidis*. The serotypes of other *S. enterica* subspecies and *S. bongori* are identified by their serotyping formula and are not given names (Grimont and Weill, 2007).

*Salmonella* spp. can be further subtyped by measuring susceptibility to a panel of bacteriophage. These types are denoted as provisional phage type (PT) or definitive phage type (DT) numbers. These two terms exist from the original two-step phage typing process between the 1950s and 1970s where a strain was originally given a PT number and later confirmed with a DT number. After the 1970s the methods were reasonably well established so the prefix PT was no longer required (Anderson *et al.*, 1977; Bell and Kyriakides, 2002). Both terms are still used in the literature.

Molecular methods are also used for *Salmonella* spp. typing in New Zealand, usually for salmonellosis outbreak or cluster investigations, and antimicrobial susceptibility is monitored. Further information on these methods, plus additional detail on serotyping and phage typing, is included in Appendix 1.

*Salmonella* Typhi and *Salmonella* Paratyphi are serotypes which cause a serious enteric fever and are particularly well adapted to invasion and survival in human tissue. They have a particular antigenic makeup and differing ecology to other serotypes of *Salmonella*. *Salmonella* Cholerae-suis (SCS) is a typhi-like serotype that infects pigs. SCS is only found

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<sup>3</sup> A full set of the data sheets can be found at:  
<http://www.foodsafety.govt.nz/science-risk/hazard-data-sheets/pathogen-data-sheets.htm>

in a few countries, excluding New Zealand and has a distinct pathogenic profile. This Risk Profile does not consider these human and porcine typhoidal serotypes.

## 2.2 Sources of *Salmonella*

The primary sources of *Salmonella* are the gastrointestinal tracts of humans and animals and its widespread presence in the environment can be considered to be due to direct or indirect faecal contamination (Bell and Kyriakides, 2002).

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

**Animal:** *Salmonella* can be found in mammals, fish, reptiles, amphibians, insects and birds. Most *Salmonella* colonisations in animals do not produce clinical signs. Some serotypes are largely confined to particular animal reservoirs causing both systemic and enteric disease, for example *S. Cholerae-suis* is host restricted to pigs (Allison *et al.*, 1969) while other serotypes (for example *S. Typhimurium*) are associated with intestinal infections in a wide range of species (Paulin *et al.*, 2002). Both plant and animal product-based animal feed ingredients may be contaminated with salmonellae. The *Salmonella* serotypes Brandenburg and Typhimurium DT9 are often associated with sporadic salmonellosis cases who have had contact with colonised animals in New Zealand (Adlam *et al.*, 2010).

**Food:** Red and white meats, meat products, milk, cheese and eggs are considered the major food sources of human salmonellosis, although a wide variety of other foods have been associated with outbreaks (Jay *et al.*, 2003). Other foods that have been contaminated by *Salmonella* include seafood (shellfish, salmon), nuts and nut products (desiccated coconut, peanut butter), cereal and cereal products (barley, cereal powder), spices (white and black pepper, paprika), oilseeds and oilseed products (cottonseed, soybean sauce, sesame seeds), vegetables (watercress, tomatoes, lettuce, potato and other salads, bean sprouts), fruit and fruit products (watermelon, melon, cider) and other miscellaneous products (chocolate, cocoa powder, dried yeast, candy). The *S. Enteritidis* types that are capable of trans-ovarian transmission into eggs are not endemic in New Zealand so this food type is likely to be of lower risk here (Lake *et al.*, 2004a). *Salmonella* contaminated tahini (a product made from crushed sesame seeds) has caused a number of outbreaks worldwide, including New Zealand and Australia (Unicomb *et al.*, 2005).

**Environment:** Salmonellae in sewage effluents or animal faeces can contaminate pasture, soil and water. They do not usually multiply in soil and waters but may survive for long periods (Bell and Kyriakides, 2002). The organism may also be dispersed in dust and aerosols

generated during the handling and processing of animals. Contamination in the environment can be spread by rodents or wild bird populations and act as a source of infection for other animals.

Transmission routes: Salmonellae may be transmitted to humans via person-to-person transmission, contaminated food or water, animal contact or from a contaminated environment. A review of non-typhoidal salmonellosis sporadic cases and outbreaks in New Zealand indicated that the important pathways for *Salmonella* infection are consumption of food, consumption of untreated drinking water and contact with sick animals (Adlam *et al.*, 2010).

## 2.3 The Food

### 2.3.1 Definitions

The specific food considered in this Risk Profile is eggs. Most eggs for human consumption are derived from hens (chickens, *Gallus gallus*), but eggs from other birds such as ostriches, ducks, and quail are also consumed. Eggs are generally marketed and consumed as shell eggs. For commercial use, and in food service operations, eggs are broken from their shells, and may then be mixed whole or separated into whites and yolks. Further processing includes pasteurisation, drying, and possibly mixing with other ingredients.

### 2.3.2 The Food Supply in New Zealand: Eggs

The Egg Producers Federation of New Zealand Inc (EPF), funded via producer levies under the Commodity Levies Act, represents the industry. The following material is from their website<sup>4</sup>.

New Zealand currently has around 150 commercial egg producers, with the largest 20 producers accounting for over 75% of total production. Since deregulation in the late 1980's the number of commercial egg producers has declined rapidly, with subsequent consolidation of market share between the various producers.

The majority of eggs produced in New Zealand are from conventional cage production systems (88%), with the rest being produced in free range or barn egg production systems. The last ten years has also seen a wider choice of egg types available, such as omega three enriched eggs.

New Zealand's estimated 3.3 million laying hens produced around 1 billion eggs in 2010. Over 85% of eggs are sold as table eggs within the domestic market, with the remainder used in the baking and catering industries. Total egg production has slowly increased over the past decade, with an increase in per capita consumption also - now around 230 eggs per person annually.

The New Zealand Egg Industry has a small, but growing, export base. At present, significant numbers of live day old chicks and fertile hatching eggs are exported to the Pacific and Oceania regions. New Zealand also exports a small amount of table eggs and egg products, mainly to the Pacific Islands. In the year ending December 2010, over 927 metric tonnes of table eggs and egg products were exported, valued at more than \$4.2 million.

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<sup>4</sup> <http://www.eggfarmers.org.nz/default.asp> accessed 9 May 2011

### 2.3.3 Imported food

Small amounts of egg in shell and egg derived products are imported into New Zealand. According to data from Statistics New Zealand, in 2010, total imports of egg in the shell, either fresh or preserved were approximately 21 tonnes, with the majority (18.6 tonnes) from the People's Republic of China. Dried egg yolk imports are mainly from Canada, with 56.7 tonnes in 2010. Dried egg white imports totalled approximately 58.5 tonnes in 2010, with the majority of product from Canada (34 tonnes) and the US (20 tonnes).

### 2.3.4 Behaviour of *Salmonella* in eggs

Eggs are made up of;

- The cuticle, a largely proteinaceous coating on the exterior of the shell;
- The shell, which is mostly calcium carbonate;
- The outer coarse membrane;
- The inner fine membrane;
- The outer thin white (albumen layer #1);
- The thick white (albumen layer #2);
- The inner thin white (albumen layer #3);
- The chalaziferous layer which anchors the yolk in the centre of the egg (albumen layer #4);
- The vitelline membrane that encloses the yolk; and
- The yolk.

(ICMSF, 2005)

The contents of eggs are high in moisture (approximately 75%) and contain nutrients that would allow bacterial growth. Barriers to prevent bacterial contamination of the contents include the physical barrier of the cuticle, shell and associated membranes, and antimicrobial components present in the egg white (albumen) and the vitelline membrane. Egg yolk (or mixtures of egg white and yolk) does not have antimicrobial activity (ICMSF, 2005).

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.

*Salmonella* can contaminate eggs internally by two routes;

- trans-ovarian - a vertical transmission; and
- trans-shell - a horizontal transmission

The mechanisms for contamination and the properties of the egg that reduce contamination of the contents have been reviewed; for trans-ovarian transmission (Gantois *et al.*, 2009) and trans-shell transmission (Messens *et al.*, 2005).

Vertical transmission results from *Salmonella* colonisation of the hen reproductive tissues and is considered by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) to be the major route of contamination and is more difficult to control (FAO/WHO,

2002). To date, results from surveys undertaken from New Zealand poultry flocks and eggs have not isolated the particular types of *Salmonella* most commonly involved in vertical transmission. Therefore incidents of salmonellosis in New Zealand where eggs are implicated are likely to be caused by external contamination of the eggs, by dirt and/or faecal material.

Most *Salmonella* infections attributed to eggs overseas are caused by *S. Enteritidis* and therefore most international research into *Salmonella* has focused on this serovar. A recent review concluded that there were not consistent differences between *S. Enteritidis* and *S. Typhimurium* in terms of the factors that affect horizontal transmission (Wales and Davies 2011). Consequently this Risk Profile uses studies on *S. Enteritidis*, with the assumption that the results are applicable to other serotypes as well.

#### 2.3.4.1 *Trans-ovarian (vertical) transmission:*

The unusual ability by certain *Salmonella* serovars to colonise and infect hen ovaries or oviduct tissues may result in transfer of salmonellae to the yolk or albumen, prior to formation of the shell or shell membranes. The mechanism for this ability is unclear, but appears to be partially linked to the possession of a particular type (SEF14) of fimbriae (proteinaceous, external appendages involved in adhesion to other cells) that allows reproductive tissue colonisation (Messens *et al.*, 2005). Other factors involved in reproductive tissue colonisation include flagellae, outer membrane lipopolysaccharides, cell wall structure and stress tolerance. These factors are not specific to *S. Enteritidis* and so it has been speculated that unique regulation of these known virulence factors could explain the association of this serotype with hen's eggs (Gantois *et al.*, 2009).

*S. Enteritidis* emerged in the late 1970s as the leading cause of human salmonellosis in many countries (not New Zealand). Although *S. Enteritidis* is the most common serovar to invade the reproductive tissues of laying hens (Humphrey, 1994), other *Salmonella* species are occasionally found in reproductive tissues as well (e.g. *S. Typhimurium* and *S. Heidelberg* (Gast *et al.*, 2007; Poppe *et al.*, 1992)).

Although there was a simultaneous increase in *S. Enteritidis* cases associated with consumption of raw eggs in both Europe and North America, in Europe this was largely associated with phage type 4 (PT4) while in North America a range of other phage types were also involved (ICMSF, 2005).

Host specific poultry pathogens, *S. Gallinarum* and *S. Pullorum* are highly adapted to the host species and can be isolated from hen reproductive tissues but are of little public health concern. These serovars are not believed to be present in the New Zealand poultry flock (Christensen, 2006; Davidson, 2002). MAF reports annually to the World Organisation for Animal Health (OIE) on the status of *S. Pullorum*, which has not been isolated in New Zealand since 1985 (*S. Gallinarum* is not reportable to OIE) (Anonymous, 2011).

#### 2.3.4.2 *Trans-shell (horizontal) transmission:*

The ability of *Salmonella* on the exterior of eggs to penetrate into the yolk where growth is most likely depends on a number of intrinsic factors related to physical barriers and chemical antibacterial factors. The contents within the egg yolk are perishable when exposed, but the protection of the shell and the antimicrobial components in the egg white that surround the

yolk allows for the uncontaminated egg to remain edible for months, even when stored at room temperature (ICMSF, 2005).

There are three physical barriers on the egg exterior: the hydrophobic cuticle, the crystalline shell itself, and membranes which separate the egg shell and the albumen (Messens *et al.*, 2005). The cuticle makes the shell resistant to the entry of water through the shell's pores, and consequently also provides protection against bacterial contamination. Immediately after laying (oviposition) the cuticle is wet and only fills the pores after a few minutes required for drying and oxidation. The cuticle has variable thickness and an uneven or patchy distribution on the eggshell surface (Board and Halls, 1973). During this time, the laid egg is susceptible to bacterial penetration (Sparks and Board, 1985). Cuticle damage and shell cracks can also allow penetration by bacteria which may cause spoilage or be pathogenic to humans.

Egg white (albumen) contains a number of antibacterial factors including lysozyme and conalbumin (also known as ovotransferrin). Lysozyme degrades the bacterial cell wall while conalbumin sequesters metal ions needed by bacteria for growth (ICMSF, 2005). Freshly laid hen's eggs have a pH of around 7.6 -7.8. Carbon dioxide is lost from the egg after lay, resulting in the pH of the egg white rising to 9.1 – 9.6 after 1-3 days of storage at room temperature. This inhibits bacterial growth, and also enhances the chelating activity of conalbumin (ICMSF, 2005).

Extrinsic factors that may affect penetration and subsequent growth of bacteria include (ICMSF, 2005):

- Washing eggs in a liquid of a cooler temperature than that of the egg may result in bacteria being drawn through the pores;
- Any process that wets the shell such as sweating and cleaning with a wet cloth;
- Dirt or faecal matter on the shell or in wash water;
- Washing techniques that damage the cuticle (e.g. through abrasion); and,
- Wash water containing iron which may reduce the protection provided by conalbumin.

These intrinsic and extrinsic factors are further discussed in Appendix 1.

### 2.3.5 Egg production and factors affecting *Salmonella* contamination

Infection of flocks is the primary reason eggs get contaminated with *Salmonella* (Zhang *et al.* 2011). *Salmonella* infection in a layer flock does not result in 100% of birds being positive for the pathogen and the percentage of excretors found in various studies is up to 30% (ICMSF, 2005). In experimentally infected birds, the percentage of eggs laid with contamination of the contents by *S. Enteritidis* is up to 28% whereas the contamination of contents in naturally infected layer flocks is mostly below 3% (De Reu *et al.*, 2008).

The prevalence of contamination on egg shells from hen faeces from experimentally inoculated birds varies over time between bird infection and laying. The prevalence of *S. Enteritidis* in the shells of eggs from hens (n=30) dosed orally with  $10^{10}$  cfu *S. Enteritidis* was 52.5% at week 1 post inoculation. The prevalence on egg shells then declined to approximately 10% at weeks 4 and 8 post inoculation (Bichler *et al.*, 1996). From hens artificially infected with *S. Senftenberg*, *S. Thompson* and *S. Typhimurium* the prevalence of shell contamination on eggs

was up to 10%. Studies of naturally infected flocks have found prevalences of shell contamination of eggs of approximately 1% (Messens *et al.*, 2005; De Reu *et al.*, 2008).

Many factors can influence the colonisation of *Salmonella* in hens including feed, water, vectors and general hygiene conditions (ICMSF, 1998).

The acquisition of birds from *Salmonella*-free parent flocks is an important means to reduce infection and layer hens should be raised under conditions that minimise stress. Short periods of environmental stress, such as water and food deprivation, infections with other pathogens and moulting have been noted as factors leading to an increased risk of *Salmonella* secretion in flocks (Howard *et al.* 2011). Stress can cause oviduct damage leading to ultra-structural defects in the eggshell, increasing the susceptibility to bacterial invasion (Nascimento and Solomon, 1991).

In Europe, a ban of conventional cage systems will take effect in 2012 and a study investigating the role of egg production systems such as caged, free range and organic found that housing in conventional battery cages was a risk factor in comparison to the other systems (Van Hoorebeke *et al.*, 2010). The large size of the battery systems, the absence of dry cleaning in between production cycles, and season (winter) were noted as risk factors for *Salmonella* infection. Probiotics and vaccination are used in some countries to prevent infection (Howard *et al.*, 2011).

Contaminated feed and water can be sources of *Salmonella* on the farm. Feed for layer hens is mostly provided as unpelleted mash, which unlike broiler feed does not receive a heat treatment during pelleting to kill pathogens. Organic acids are often used to reduce contamination in the mash, which has the advantage of protecting feed against recontamination during storage and distribution. Open troughs of drinking water can also 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 mash (estimated 60-65% of production uses mash). Pelleted feed is heat treated (>70°C) during production, which controls *Salmonella* contamination. Mash is simply mixed and stored in bags at ambient temperature. *Salmonella* inhibitors (organic acids) may be added to mash as required (Dr James Fick, Egg Producers Federation, pers. comm. 28 July 2011).

Pathogen transmission can be limited by a variety of on farm environmental measures including cleaning and disinfecting houses between successive flocks, controlling the movements of people and equipment on the farm as well as limiting hen exposure to rodents, insects, birds and other animals which may carry *Salmonella*. Such control measures can reduce the potential of introducing the organism into the flock or transmitting it among existing flocks to and from other flocks (Howard *et al.*, 2011). However, in practice such measures may not be effective. A study in the UK found that *Salmonella* strains could be isolated from flocks over more than one cycle, and could be detected by sampling after cleaning and disinfection (Carrique-Mas *et al.*, 2009).

Egg collection on small farms is often by hand. Semi-automated collection systems are common in caged hen eggs and have been reported to produce lower contamination rates than eggs laid into nests. In addition, shorter times between laying and collection of the eggs

resulted in lower contamination rates, presumably due to reduced opportunity for contamination from the environment (ICMSF, 2005).

When given favourable conditions and time, *Salmonella* present on the egg shell can penetrate the egg and grow. The likelihood and rate of penetration of bacteria into the contents is influenced by intrinsic factors relating to the egg and extrinsic factors found externally to the egg. How the eggs are handled or treated through the production chain (extrinsic factors such as temperature, moisture, presence of faecal matter and washing techniques) can affect the integrity of the intrinsic factors (cuticle, shell, albumen, yolk etc.) that protect the egg from penetration.

The survival of *S. Enteritidis* on the shell surface is relatively short (a few days) when applied as an aqueous suspension (Baker, 1990). Survival was reported to be better at 7°C than it was at room temperature. Low temperatures and high relative humidity enhance survival on the shell (Messens *et al.*, 2005). *Salmonella* in faecal material inoculated onto egg surfaces have been shown to penetrate the shell and was isolated from eggs contents within 3 days at 25°C, but not at 4°C. The highest percentages of contaminated eggs and fastest penetration rates were found when a temperature differential is encountered by the microorganism in situations such as when the eggs are transferred from the hatchery to storage (Schoeni *et al.* 1995). *Salmonella* were also found to survive longer at 21°C on eggs contaminated with faeces compared to clean eggs (Braun *et al.* 1999). Therefore, the rapid removal of faecal contamination on the eggshell could potentially decrease *Salmonella* penetration into the egg (Schoeni *et al.*, 1995).

*Salmonella* may also contaminate eggs during collection and packing procedures. 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. Cross contamination of *Salmonella* can also occur through manual handling or from processing equipment. *S. Enteritidis* has been found to be common on equipment and surfaces in egg packing areas on UK farms where flocks were infected with this bacterium (Davies and Breslin, 2003). Although, it is unrealistic to achieve complete eradication of *Salmonella* in the process chain, cleaning and disinfection procedures are essential to reduce the prevalence of *Salmonella* in these key areas.

The use of egg washing is controversial despite its broad commercial application, due to concerns that it can increase the internal microbial load (Zhang *et al.*, 2011). Egg washing is widely used in many countries, including some producers in Australia (Hutchison *et al.*, 2004), and is required in the US and Canada (ICMSF, 2005). However, 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. Wet washing may also damage the cuticle layer increasing the risk of bacterial penetration (Chousalkar *et al.*, 2010).

The Technical Annex of the Egg Producers Federation of New Zealand Code of Practice (Egg Producers, 2002) reports that some producers in New Zealand practice egg washing. Factors relating to washing procedures and the microbial penetration and spoilage are listed in the Annex. Overall, the Code of Practice advises that washing should only be performed if it can be carefully controlled and in accordance with the guidelines provided.

The Code of Practice also describes the requirements for transportation and storage. These parts of the production chain can be prone to undesirable changes, such as temperature fluctuations which in turn could provide favourable conditions for the penetration and growth of *Salmonella*. The Code specifies that eggs be transported to the grading room, or stored in

cool rooms operated at or below 15°C within 2 hours of collection. Cracked eggs are to be stored at or below 6°C and must undergo further processing, such as pasteurisation or equivalent. The additional processing inactivates any potential *Salmonella* that may be present in the egg and they can therefore be used in other products. 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.

Further detail is provided in Appendix 1.

## 2.4 Exposure Assessment

### 2.4.1 Salmonella in eggs

The 2004 Risk Profile outlines details of surveys performed in 1994 and 2001 that investigated the prevalence of *Salmonella* in eggs in New Zealand. Briefly, the survey performed in 1994 reported that *Salmonella* was not detected on the shells (n=2037) or contents of eggs (n=2037) (Johnson, 1995), while the 2001 survey, which was performed alongside a case-control study (Thornley *et al.* 2002), reported the isolation of *Salmonella* from the surface of 14% (13/93) egg samples (Maurice Wilson, ESR, pers. comm.)

A survey carried out by ESR in 2007 assessed the presence of *Salmonella* in and on eggs available through retail outlets in Auckland and Christchurch (Wilson, 2007). A total of 514 sample units (consisting of a retail pack of at least 6 eggs) were tested over a twelve-month period, representing cage, free range and barn production systems. The proportion of samples from each production system was targeted at 50% cage laid, 30% free-range and 20% barn laid. Forty nine different brands or sub-brands were tested. *Salmonella* was isolated from nine shell surface samples (1.8% of sample units of 6 eggs, from which one was tested for surface contamination). All positive samples were from cage laid eggs, and all isolates identified as *S. Infantis*. No egg contents (3,710 eggs) were positive for *Salmonella*. Of the egg samples that tested positive for *Salmonella*, 4 out of 9 sample units contained “dirty” eggs (obvious contamination of shell with faecal, feather or other organic material).

With some exceptions, the prevalence of *Salmonella* on the surface of eggs in overseas surveys is as low, or lower, than the most recent survey in New Zealand (see Appendix 1). The prevalence of *Salmonella* internally is generally lower than on the surface, and in many surveys no internal contamination was found, as in New Zealand. The prevalence of *Salmonella* in eggs appears to vary markedly over time in some countries. For example, results from a series of surveys in the UK show *Salmonella* contamination rates spiked in 2005-2006, but this increase was due to the contamination in Spanish eggs that were imported into the UK. Such occasional spikes in contamination may also occur in New Zealand, as suggested by the case-control investigation into an outbreak of *S. Typhimurium* DT160 (Thornley *et al.*, 2002).

### 2.4.2 Food consumption: Eggs

The World Health Organisation Global Environmental Monitoring System (GEMS)/Food regional diets give a range of egg consumptions from 3.7 g/person/day (African diet) to 37.6 g/person/day (European diet)<sup>5</sup>. There are only five GEMS/Food regional diets and New Zealand, Australia, Canada and the US are represented by the European regional diet.

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<sup>5</sup> [http://www.who.int/foodsafety/publications/chem/regional\\_diets/en/](http://www.who.int/foodsafety/publications/chem/regional_diets/en/)

The more recent GEMS/Food cluster diets group New Zealand with Australia, North America and southern South America, with an average chicken egg consumption of 34.4 g/person/day.<sup>6</sup> The cluster diets represent groupings of countries with similar food consumption patterns.

The New Zealand Egg Producers Federation website states that the annual egg consumption per capita in New Zealand is 230 (4.4 eggs/week)<sup>7</sup>. The Concise New Zealand Food Composition Tables<sup>8</sup> give the weight of a medium (size 5), standard (size 6) and large (size 7) egg as 43, 53 and 56 g, respectively. Based on the weight of a standard egg, 230 eggs per annum equates to 33.4 g/person/day.

At the time of preparing this Risk Profile, the National Nutrition Survey (NNS) results from 2008-09 were not yet published; therefore figures from the 1997 survey were used. An analysis of the 24-hour dietary recall data from the 1997 NNS (Russell *et al.*, 1999) using standard recipes to identify egg containing foods, concluded that 80.3% of respondents ( $n = 4,636$ ) consumed eggs in the previous 24-hour period (ANZFA, 2001). The analysis estimated the mean daily consumption of eggs for all respondents as 23.2 g/person/day.

However, the majority of these 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, 22% of the respondents reported consuming such dishes in the previous 24 hours. The most common consumption forms were:

- Eggs, whole, boiled 31%
- Eggs, whole, fried 28%
- Eggs, whole, poached 16%
- Eggs, scrambled 9%
- Eggs, whole, omelette 5%

According to 1997 NNS dietary recall records, a very small number of servings (7 out of 1,031, 0.7%) were for whole raw eggs. It is possible that there is consumption of raw eggs as a component of mayonnaise or similar products. The 1997 NNS 24-hour dietary recall records include 47 records involving consumption of homemade mayonnaise, of which four are reported as containing eggs. A single record of each of hollandaise sauce and egg custard are also reported in the 1997 NNS, but without any indication of whether they were home or commercially prepared.

A study of foods consumed by 12-24 month old New Zealand children found that 32% of respondents consumed eggs on at least one of three non-consecutive survey days (Szymlek-Gay *et al.*, 2010). The median daily quantity of egg consumed by those reporting consumption was 25 g.

Australian statistics indicate lower levels of egg consumption in that country, with 16.7% of the population 19 years and over consuming eggs or egg-based dishes in any 24 hour period. The average consumption of eggs was 13.7 g/person/day or 137 eggs per capita (2.6 eggs /week) (ABS, 2000). The UK Family Food Survey for 2009 gives a similar estimate of average

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<sup>6</sup> <http://www.who.int/foodsafety/chem/gems/en/index1.html>

<sup>7</sup> <http://www.eggfarmers.org.nz/industry-statistical-facts.asp>

<sup>8</sup> [http://www.crop.cri.nz/home/products-services/nutrition/foodcompdata/fcd-products/Concise\\_8\\_Edition.pdf](http://www.crop.cri.nz/home/products-services/nutrition/foodcompdata/fcd-products/Concise_8_Edition.pdf)

household consumption of 104 eggs per capita (2 eggs /week) (DEFRA, 2011). US egg consumption is similar to that for New Zealand. The US Poultry and Egg Association (USPEA) estimate annual egg consumption in the US to be 246.2 egg per capita (4.7 eggs/week) (USPEA, 2011). This was calculated from total egg production divided by total population. Egg consumption has been declining in the US since 2004 where consumption was estimated to be 257.1 (4.9 eggs/week) per capita, this appears to be partially due to publicity of the cholesterol content of eggs.

A 2009 survey of Australian consumer behaviour and egg consumption conducted for the Primary Production Standard for eggs by FSANZ found that 81% ( $n = 4,616$ ) of Australians reported buying eggs from supermarkets, while 10% bought eggs from “other retail stores” (e.g. fruit and vegetable shop, butcher, corner shop, etc.) and 11% from farmers markets. Back yard producers and “own chickens” were reported as egg sources by 9% and 5% of consumers respectively (FSANZ, 2009).

### 2.4.3 Evaluation of exposure

#### 2.4.3.1 *Number servings and serving sizes*

The number of servings and serving sizes of eggs in New Zealand can be calculated using figures from the 1997 NNS (Russell *et al.*, 1999; ANZFA, 2001). In this survey, 3,722 respondents out of a total of 4,636 reported consumption of eggs in the previous 24-hour period. This includes eggs consumed as ingredients in standard recipes as defined by the Australia New Zealand Food Authority (ANZFA) analysis. Assuming consumption of a single serving of eggs per day and using a total New Zealand population of 4,000,000, the following can be calculated:

$$\begin{aligned} \text{Annual number of servings (total population)} &= 3722 \times 4,000,000 / 4636 \times 365 \\ &= 1.17 \times 10^9 \text{ servings} \end{aligned}$$

This represents a high number of servings, as would be expected from a commonly consumed food such as eggs.

The mean egg serving size from the 1997 NNS was 28.9 g (approximately half a standard egg), while the 97.5<sup>th</sup> percentile serving size was 138.1 g (just over two large eggs) (ANZFA, 2001).

#### 2.4.3.2 *Frequency of contamination*

Data for New Zealand from two surveys (2007 survey-3,710 eggs and 2001 Auckland survey-558 eggs) indicate that contents of shell eggs are rarely if ever contaminated by salmonellae. The data for the exterior of shells from the two existing surveys are quite different, with the most recent survey indicating a contamination rate of 1.8%.

#### 2.4.3.3 *Potential growth during storage and recommended storage conditions.*

Bacteria contaminating the outside of shells are unlikely to grow as nutrients and moisture levels would be too low on cleaned eggs. *Salmonella* survive on cleaned eggs for only a few days.

The NZFSA risk management programme template contains agreed storage and shelf-life options based upon then current industry practice outline in the Egg Producers Code of Practice (2002) and on the findings of a range of scientific studies. The options for storage for eggs from the date of lay as specified in the template include:

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

There is no requirement for retailers to store eggs under temperature controlled conditions.

Information about domestic storage times of eggs by New Zealand consumers is limited. A survey of Auckland consumer knowledge of food safety issues (Bloomfield and Neal, 1997) found that 75.7% (333/440) of respondents believed that fresh eggs should be stored in the refrigerator, while 20.7% thought the cupboard shelf was suitable, and 3.6% were unsure. This finding was similar to the results from earlier studies that addressed the same issue. Refrigerated storage was identified as an appropriate means to store eggs by 71% (174/244) of respondents in a Canterbury survey (Hodges, 1993) and 56% (131/234) of respondents in a Wellington postal survey (Kerslake, 1995). The authors of the Auckland study considered that refrigerated storage should be recommended and that this was one area of food safety knowledge that could be improved.

In 2008, 1,000 people in Victoria, Australia completed an internet survey about egg buying, storage and preparation<sup>9</sup>. Results from that survey showed that 87% of respondents store their eggs in the fridge, which is similar to the 93% estimate found by FSANZ in a 2009 survey.

The FAO/WHO risk assessment reported US and Canadian data for average retail and consumer storage temperatures of 7°C, with times of 5.9 – 7 days (retail) and 6.8 – 12 days (consumer) (FAO/WHO, 2002).

If *Salmonella* on the surface of eggs do penetrate into the egg, the potential for growth depends markedly on the storage temperature. The time for yolk membrane breakdown (Yolk Mean Time or YMT) is approximately 60 days at 7°C, whereas at 20°C it is approximately 17 days. Once these times have elapsed, growth inside the egg will be rapid at higher temperatures (0.0015 log<sub>10</sub> cfu per hour at 7°C, 0.14 log<sub>10</sub> cfu per hour at 20°C). These estimates were calculated using the US equations reported in the FAO/WHO risk assessment (FAO/WHO, 2002). In the draft Australian Quantitative Risk Assessment model for *Salmonella* it was estimated the YMT for eggs stored at 16°C is 26 days and for those stored at 20°C, 17 days (Daughtry *et al.*, 2005).

The Australian Risk Profile used an estimate of the proportion of servings (an egg is considered one serving) of commercial eggs that had not undergone pathogen growth (i.e. no resolution of YMT) of 75% of total egg production (Daughtry *et al.*, 2005).

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<sup>9</sup> <http://www.health.vic.gov.au/eggs/research.htm#pre>

#### 2.4.3.4 Heat treatment

The 2009 survey of Australian consumer behaviour and egg consumption conducted for the Primary Production Standard for eggs by FSANZ reported that of all the eggs consumed, 71% and 3% of all eggs consumed during the survey were either ‘lightly cooked’ or ‘raw’, respectively. The category of ‘lightly cooked’ included eggs and egg dishes where the yolk and/or albumen (egg white) remained runny (FSANZ, 2009). An Australian Risk Profile estimated the following heat treatments for servings of commercial eggs (Daughtry *et al.*, 2005):

- None (raw egg drinks, some desserts): 7.5%
- Light cooking (boiled, fried “sunny side up”, microwave): 27.5%
- Medium cooking (fried “over easy”, lightly scrambled or omelette, pasta): 32.5%
- Heavy cooking (hard boiled or scrambled cakes, biscuits): 32.5%

D times for *S. Enteritidis* in intact eggs have been reported as 4.5 and 6.0 minutes at 58 and 57°C respectively (Schuman *et al.*, 1997). However, times and temperatures for the various methods of cooking eggs are not available, and are likely to vary considerably. The 2002 FAO/WHO risk assessment reports USDA and Health Canada estimates of log reductions of *S. Enteritidis* during frying, scrambling and boiling of eggs. The most likely log<sub>10</sub> cfu reduction estimates were 4, 6, and 1 respectively for each cooking method respectively, although the range varied from 0 to 7 log<sub>10</sub> cfu.

#### 2.4.3.5 Exposure summary

Eggs are a frequently consumed food in New Zealand. The very high number of servings and number of eggs consumed means that even a low prevalence of contamination and low frequency of improper handling and temperature abuse may present a significant risk of infection.

Most egg servings will be cooked before consumption, although the times and temperatures are not available to estimate *Salmonella* reduction. Australian estimates that 3–7.5% of egg servings are consumed raw are higher than data from the New Zealand NNS which suggest perhaps 1% of servings of raw eggs.

The YMT estimate of 60 days at 7°C is considerably longer than estimated refrigerated storage times from overseas assessments. An estimate of domestic refrigeration storage times for eggs is not available for New Zealand. Australian data shows approximately 90% of survey respondents store eggs in the refrigerator in domestic homes.

The 1.8% prevalence of *Salmonella* on egg shells in New Zealand from the most recent survey (2007) is higher than has been reported from most other overseas surveys (Appendix 1) although the most recently reported Australian survey, from Adelaide, found a prevalence on egg shells of 3.5% (Fearnley *et al.*, 2011). There is no evidence for internal contamination of eggs with *Salmonella* in New Zealand surveys conducted to date. This result may be an artefact of the low sample numbers taken for the surveys being too low to detect a very low prevalence.

## 2.5 Overseas context

A summary of overseas surveys of eggs, egg products and layer hens for *Salmonella* is given in Appendix 1.

### 3 EVALUATION OF ADVERSE HEALTH EFFECTS

#### 3.1 Disease characteristics

Information regarding the disease characteristics of non-typhoidal *Salmonella* outlined below is primarily from D'Aoust and Maurer, (2007), Jay *et al.* (2003), FAO/WHO, (2002) and the NZFSA datasheet<sup>10</sup>, unless referenced elsewhere.

*Incubation:* 8-72 hours, commonly 12-36 hours.

*Symptoms:* Non-bloody diarrhoea, abdominal pain, vomiting, nausea and fever lasting 2-7 days.

*Condition:* Salmonellosis, presents with symptoms of gastroenteritis or enterocolitis.

*Toxins:* Toxins are not produced in foods, but salmonellae may produce enterotoxins and cytotoxins within epithelial cells (Jay *et al.*, 2003).

*People Affected:* Anyone can be infected, but the young, old, and immunocompromised are particularly at risk of severe outcomes (FAO/WHO, 2002; Gorden 2008).

*Treatment:* The infection is usually self-limiting. Uncomplicated gastroenteritis may require supportive therapy such as fluid and electrolyte replacement, especially in the elderly or young children. The use of antibiotics is not recommended for mild or moderate cases because it prolongs the carriage and excretion of salmonellae.

*Long Term Effects:* Extra-intestinal infections can occur that usually involve hospitalisation and treatment with antimicrobials. An increased risk of blood stream infections (bacteraemia) has been linked to patients also having systemic lupus erythematosus, liver cirrhosis, solid organ cancers or immunodeficiency, and risk factors for atherosclerosis predisposed patients with blood stream infections to acquire endovascular infection (Hsu and Lin, 2005). Reactive arthritis may follow 3-4 weeks after onset of gastrointestinal symptoms and when it occurs can persist for 3-5 months, although long-term chronic conditions such as Reiter's Syndrome, septic arthritis or septicemia can also develop in some cases (Hannu *et al.*, 2006).

#### 3.2 Dose-Response

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). Dose-response can be calculated from human feeding trials, animal trials, *in vivo* experiments, modelling or analysis of outbreak data. Calculation of dose-response can be difficult due to differences in host susceptibilities (e.g. individuals who are young, elderly, pregnant or immunocompromised are typically more susceptible to infection) and in *Salmonella* serotype infectivity (Bollaerts *et al.*, 2008).

For *Salmonella* the dose-response relationship can be estimated from either feeding trials with volunteers, or from outbreaks where the number of cells ingested can be estimated. Using outbreak data, FAO/WHO produced a dose-response model as an output from the joint risk

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<sup>10</sup> <http://www.foodsafety.govt.nz/elibrary/industry/non-typhoid-salmonellae.pdf>. Accessed 7 November 2011.

assessments of *Salmonella* in eggs and broiler chickens (FAO/WHO, 2002). The FAO/WHO model has been developed further to account for differences in host susceptibility, serotype infectivity and food matrix (Bollaerts *et al.*, 2008).

Most recently (Teunis *et al.*, 2010) used data from 35 salmonellosis outbreaks, three sporadic cases for which there was good dose information and two human volunteer feeding studies to estimate that the number of cells that need to be ingested to cause a 50% probability of illness was 36.3, although the 95% percentiles were wide (0.69-1.26x10<sup>7</sup>).

Further details are given in Appendix 2.

### 3.3 New Zealand Outbreak Information and Human Health Surveillance

Salmonellosis is a notifiable disease in New Zealand. The number of cases and incidence of notified (non-typhoidal) salmonellosis since 2003 is shown in Table 2.

**Table 2: Incidence data for salmonellosis in New Zealand**

Year	Number of cases	Incidence (cases/100,000)
2003	1,401	34.8
2004	1,080	26.4
2005	1,383	33.7
2006	1,335	31.9
2007	1,274	30.1
2008	1,346	31.5
2009	1,129	26.2
2010	1,146	26.2

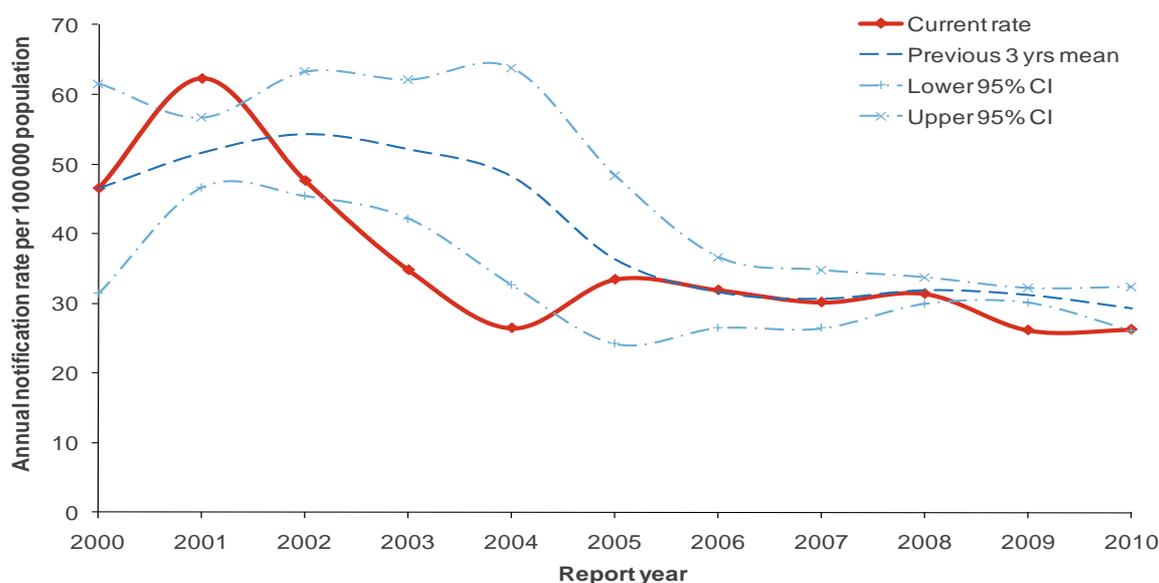
Number of cases data taken from (ESR, 2011), Population data for June each year taken from ([http://www.stats.govt.nz/methods\\_and\\_services/access-data/tables/national-pop-estimates.aspx](http://www.stats.govt.nz/methods_and_services/access-data/tables/national-pop-estimates.aspx)). Due to population adjustments by Statistics New Zealand rates may differ slightly from older Annual Surveillance Summary reports.

The notification rate per 100,000 population for cases of salmonellosis in New Zealand from 2000 – 2010 is shown in Figure 4. The rate has been stable since 2005 at approximately 30±4 per 100,000.

The incidence of salmonellosis is characterised by a late summer peak and a winter trough. Rates of salmonellosis vary throughout the country but higher rates are often reported from the lower South Island, in particular South Canterbury District Health Board (DHB) (2010 rate was 66.2 cases per 100,000, 37 cases) features in the highest quantile of salmonellosis notification rates between 2008 and 2010.

Reported rates are similar for males (26.2/100,000 in 2009) and females (25.7/100,000 in 2009). Age specific rates are highest for the <1 year age group (123.7/100,000 in 2009), and 1 to 4 year olds (89.9/100,000 in 2009).

**Figure 2: Incidence of notified salmonellosis in New Zealand 2000 – 2010**



Reproduced from (Lim *et al.*, 2011).

### 3.3.1 Clinical outcomes: Salmonellosis in New Zealand

Hospitalisation and fatality rates for notified cases of salmonellosis in New Zealand are given in Table 3. These outcomes are not always reported for each case, so percentages are expressed in terms of the number of cases for which outcomes are known. The hospitalisation rate and number of deaths has been stable over many years.

**Table 3: Outcome data for salmonellosis in New Zealand, 2005-2009\***

Year	Hospitalised cases	Fatalities
2005	142/1134 (12.5%)	1/1383 (0.07%)
2006	148/1111 (13.3%)	1/1335 (0.07%)
2007	110/833 (13.2%)	1/1274 (0.07%)
2008	123/896 (13.7%)	1/1346 (0.07%)
2009	134/716 (18.7%)	1/1129 (0.09%)
2010	136/763 (17.8%)	0/1146 (0%)

\*Data taken from annual reports on notifiable diseases in New Zealand available from: [http://www.surv.esr.cri.nz/PDF\\_surveillance](http://www.surv.esr.cri.nz/PDF_surveillance)

Chronic sequelae of *Salmonella* infections include reactive arthritis. A study carried out in the south of New Zealand found evidence of preceding *Salmonella* infection in two of 60 (3.3%; 95<sup>th</sup> percentile confidence interval 0.4-11.5%) cases of reactive arthritis (Highton and Priest, 1996). Studies from other countries have found the rates of *Salmonella*-associated reactive arthritis to vary from 4.2-18.7% (Townes 2010).

### 3.3.2 Serotypes causing disease in New Zealand

The Enteric Reference Laboratory (ERL) performs typing of *Salmonella* for the whole of New Zealand. From 2000 through 2009, *S. Typhimurium* was the most prevalent serotype reported

for salmonellosis cases in New Zealand (Adlam *et al.*, 2010). This serotype caused 58.2% of 11,554 cases for which serotype information was available. The next most frequently reported serotype over this period was *S. Enteritidis* (8.8% of cases). When considering serotype and phage type, *S. Typhimurium* DT160 was most frequently reported (19% of cases). There were 35 serotypes that caused 50 or more salmonellosis cases during this period, and together these serotypes caused 80% (9,290) of the 11,554 cases.

The incidence of the five serotypes causing the most number of cases from 2000 through 2009 (*S. Typhimurium* DT160, *S. Typhimurium* DT1, *S. Brandenburg*, *S. Typhimurium* DT135 and *S. Typhimurium* DT156) all peaked during 2000 through 2002 (Adlam *et al.*, 2010). While these serotypes are still isolated frequently from salmonellosis cases (*S. Typhimurium* DT160 is still the most commonly isolated serotype), a variety of other serotypes have peaked in recent years, such as *S. Infantis*, *S. Mbandaka* and *S. Stanley*.

The serotypes significantly associated with cases living in highly urban areas are *S. Infantis* ( $p < 0.001$ ) and *S. Typhimurium* DT160 ( $p < 0.05$ ) (Adlam *et al.*, 2010). The serotypes significantly associated with cases living in highly rural areas are *S. Saintpaul* ( $p < 0.001$ ), *S. Brandenburg* ( $p < 0.01$ ) and *S. Typhimurium* DT101 ( $p < 0.05$ ).

Between 2000 and 2010, 1332 isolates of *S. Enteritidis* (8.2%) were cultured from the 16,179 unique cases of salmonellosis recorded in EpiSurv (Muriel Dufour, ESR Enteric Reference Laboratory, pers. comm., June 2010). Of these, 118 isolates were of *S. Enteritidis* PT4 (0.7%), which has been associated with transovarian transmission, particularly in Europe. Most of these cases (68/118) were overseas during the incubation period and so probably acquired the infection overseas. For the remaining cases, 26/118 were not overseas during the incubation period, while the status of the remaining 24 cases was unknown. This supports the conclusion that domestically acquired infection with this type of *Salmonella* is rare in New Zealand.

Appendix 2 contains more detail on *Salmonella* serotypes of human isolates in New Zealand.

The ERL also receives isolates of *Salmonella* for typing from laboratories which have isolated them from non-human samples, including food products, animal feed, and food production environment. Between 2005 and 2009, 273 isolates of *Salmonella* from poultry “product” were provided and typed. Of these, 14 were from eggs (6 *S. Infantis*, 3 *S. Anatum* 15+, 2 *S. Thompson*, 1 *S. Brandenburg*, 1 *S. Typhimurium* PT160, 1 *S. species* 6,7:k:-) (Muriel Dufour, ERL, pers. comm., 13 May 2011). Although *S. Enteritidis* has not been isolated from eggs to date, the sampling and provision of isolates for this programme is not systematic or consistent.

### 3.3.3 Antimicrobial resistance of New Zealand *Salmonella* strains

ESR (Antibiotic Reference Laboratory) tested 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 results of this testing have been compiled in Appendix 1 for the years 2005 through 2009.<sup>11</sup> An analysis for the period 2002-2007 concluded that although there were trends of increasing rates of antibiotic non-susceptibility in *Salmonella* in New Zealand, the rates were still lower than in many international settings (Broughton *et al.*, 2010).

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<sup>11</sup>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 1 December 2010).

### 3.3.4 Outbreaks

The number of reported outbreaks of salmonellosis in recent years in New Zealand is given in Table 4 (figures exclude *S. Typhi* and *S. Paratyphi*). The number of cases reported as outbreaks is approximately 10% of those reported as sporadic cases. As a proportion of enteric outbreaks or cases, salmonellosis makes a small contribution; the outbreak data are dominated by reported outbreaks of norovirus.

**Table 4: Reported outbreak data for salmonellosis in New Zealand 2005-2010 (as a proportion to total enteric bacterial, viral, parasitic and gastroenteritis outbreaks and cases)**

Year	Salmonellosis outbreaks/ total enteric outbreaks	Outbreak Cases/Total Enteric Outbreak Cases*
2005	26/338 (7.7%)	120/2343 (5.1%)
2006	22/481 (4.6%)	74/6162 (1.2%)
2007	8/477 (1.7%)	141/7821 (1.8%)
2008	15/428 (3.5%)	163/6295 (2.6%)
2009	12/586 (2.0%)	76/10176 (0.7%)
2010	23/559 (4.1%)	100/5929 (1.7%)

\* Includes both suspected and confirmed cases. Data taken from annual reports on outbreaks in New Zealand available from: [http://www.surv.esr.cri.nz/PDF\\_surveillance](http://www.surv.esr.cri.nz/PDF_surveillance)

An ESR review of 204 salmonellosis outbreaks from 2000-2009 found that while non-typhoid salmonellosis was primarily a foodborne disease in New Zealand, there was insufficient information to identify important food vehicles (Adlam *et al.*, 2010; King *et al.*, 2011). Of the 70 outbreaks with at least some evidence of the mode of transmission, 24 had “moderate” evidence, and eggs were suspected as a vehicle in 3 of the 23 (13%) outbreaks in which a suspected food was reported, and for a further 3 outbreaks both chicken and eggs were suspected. Of the 24 outbreaks with “strong” evidence, infected food handlers were identified in two outbreaks, although suspected food vehicles involving eggs were also reported, while a food source involving eggs was identified in another two outbreaks. These results are summarised in Table 5.

**Table 5: Salmonellosis outbreaks in New Zealand from 2000-2009 where foods involving eggs were suspected vehicles/sources**

Outbreaks in which an infected food handler was identified								
Setting	Year	Salmonella serotype	No. cases <sup>1</sup>			Suspected source(s)	Other contributing factors	
			Conf	Prob	Exp			
Afternoon tea function	2001	Brandenburg	11	10	55	Egg and salmon sandwiches	Cross-contamination	
Restaurant <sup>2</sup>	2002	Typhimurium DT 160	4	0	?	Chocolate mousse containing raw egg	Inadequate cooling/refrigeration Use of ingredients from unsafe sources	
Outbreaks in which a food vehicle was identified								
Setting	Year	Salmonella serotype	No. cases <sup>1</sup>			Confirmed source(s)	Evidence	Other contributing factors
Home	2001	Typhimurium DT 160	2	0	2	Raw egg mayonnaise	Salmonella isolated from mayonnaise	(none reported)
Café/bakery	2005	Thompson	9	4	13	Chicken sandwich, bacon and egg pie, panini, fried chicken, chicken roll	Salmonella isolated from food (does not specify which food) and family member of food handler	Improper storage prior to preparation Improper cooling or refrigeration Cross contamination

1. Conf., confirmed cases, Prob., probable cases; Exp., exposed people; ?, unknown (data not available).

2. While two food handlers were carriers of Salmonella, both had also consumed the chocolate mousse so it could not be ascertained if the illness was caused by temperature abuse of the mousse or contamination by a food handler (or both). There is no information to indicate that the chocolate mousse was tested.

### 3.3.5 Case-control studies and risk factors

To date, seven case-control studies of salmonellosis have been conducted in New Zealand. Two of these have examined eggs as a potential risk factor for salmonellosis and were described in the 2004 Risk Profile (Lake et al. 2004a) in greater detail. The first study involved *S. Typhimurium* DT160 and was prompted by a marked increase in the number of DT160 human isolates appearing in 2000 (Thornley *et al.*, 2003). In this study, various exposure routes as well as egg consumption was examined in detail (a variety of egg dishes as well as derived foods such as mayonnaise and custard) but none of the egg related risk factors had a statistically significant odds ratio.

The second case-control study was conducted by ESR in late January 2002, as a component of the NZFSA quantitative risk assessment of *Salmonella* in New Zealand sheep meat (NZFSA, 2002). Consumption of eggs in general was protective for salmonellosis (OR 0.45, 95% CI 0.26-0.74). The odds ratios for more detailed analyses (cooked eggs, raw eggs, homemade food with raw eggs, and homemade food with cooked eggs) were not significant.

Five additional case-control studies have been conducted into outbreaks of infection with a single *Salmonella* serotype, but egg consumption was investigated as a risk factor in only one of these studies (McCallum and Das, 2008).

A case-control study into a 2008 outbreak of *S. Mbandaka* infection did not conclusively identify a causative food (McCallum and Das, 2008). There were increased risks of infection associated with purchasing chicken breast from a supermarket that was supplied by a specific poultry processor, and eating eggs prepared away from home. The results suggested that there was also an association between lettuces and chicken purchased from the supermarket, however the authors reasoned that this outcome may be due to consumers being more likely to purchase both items. An environmental investigation tested food samples from cases homes and implicated food premises, plus swabs from bench tops, chopping boards, fridges and hand wash basins. *Salmonella* was not isolated from any food or environmental samples. During the outbreak, *S. Mbandaka* was isolated from samples taken as part of routine monitoring of poultry feed, poultry products and the poultry processing environment. *S. Mbandaka* had been isolated from these types of samples in previous years, but at lower prevalence.

Further details on these case-control studies are provided in Appendix 2, Section 10.2.

#### 3.3.5.1 *New Zealand attribution studies*

In 2007 the NZFSA Science Group reported on modelling activities to support decision making on importing poultry products from the UK<sup>12</sup>. The initial phase of this work involved estimating the number of salmonellosis cases per year attributable to different exposure pathways. Expert opinion predicted an estimated 9,000 cases of human salmonellosis per annum in New Zealand, of which 63% (5,668) were estimated to be caused by foodborne transmission. Epidemiological reports of cases in NZ (1998-2003) and Australia (1995-2000)

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<sup>12</sup>NZFSA Science Group Report. 2007. Modelling of exposure of New Zealand consumers to Salmonella. Access via website: [http://www.foodsafety.govt.nz/elibrary/industry/Modelling\\_Exposure-Evaluates\\_Relative.pdf](http://www.foodsafety.govt.nz/elibrary/industry/Modelling_Exposure-Evaluates_Relative.pdf). Accessed 7<sup>th</sup> November 2011

were used to estimate the contribution of different food categories. Eggs were estimated to contribute 595/5,668 (10.5%) of foodborne cases, or 595/9,000 (6.6%) of all salmonellosis cases (taking into account non-foodborne pathways). Additional analyses, based on the prevalence of different *Salmonella* serotypes isolated from foodstuffs and from human cases from 2002 through 2004, estimated the relative contribution of selected food groups.

The largest proportion of salmonellosis cases were attributed to chicken, and while this proportion declined each year, it remained significantly higher than the proportions of salmonellosis cases attributed to the other food groups (beef/veal, pork, lamb/mutton, eggs).

A New Zealand study using molecular sub-typing data and Bayesian techniques ('modified Hald model') estimated the attributable food source for human salmonellosis cases in New Zealand in 2003 (Mullner *et al.*, 2009). The majority of cases were attributed to pork (60%), followed by poultry (21.2%). The authors advised caution in interpreting the results for pork because the data for pork were sparser and more biased than data for other sources. Eggs (3.2%) and lamb and mutton (1.4%) were estimated to be minor sources of infection.

A systematic review of the aetiology of salmonellosis in New Zealand was reported in 2009 (Wilson and Baker, 2009). This concluded that contaminated food was "very likely" (>90% probability) to be "the majority cause" (i.e., >50% of all cases). This conclusion was largely based on data from the case-control study, the outbreak data and the comparisons of serotypes. Of the foods considered, poultry was considered "most likely" to be a moderate cause (10-30% or more of cases), while pig meat, beef, and meat in general were considered to be "likely" to cause a similar number of cases. Eggs were considered "likely" (>66% probability) to be a minor cause (<10%) of foodborne cases).

A later review of 204 New Zealand salmonellosis outbreaks from 2000 through 2009 was not able to quantify the proportions of salmonellosis cases attributable to specific foods (Adlam *et al.*, 2010; King *et al.*, 2011). There were only eight outbreaks where specific foods were identified by laboratory evidence as being contaminated with *Salmonella*; two of these foods contained eggs, but in one case the record did not identify which of a variety of bakery products (chicken sandwich, bacon and egg pie, panini, fried chicken, chicken roll) was *Salmonella*-positive. For the other outbreak, raw egg mayonnaise was identified as the causative food.

### 3.4 Adverse Health Effects Overseas

The incidence of notified cases of salmonellosis in New Zealand is similar to rates in other developed countries, particularly Canada and Australia. In contrast to New Zealand, in the EU the dominant serotype is *S. Enteritidis*. A number of outbreaks of *S. Typhimurium* associated with eggs have occurred in Australia over the last ten years (see Appendix 2).

Several case-control studies overseas have identified significant associations between salmonellosis (particularly infection with *S. Enteritidis*) and consumption of products containing raw eggs (Banatvala *et al.*, 1999).

### 3.5 Health Burden of Infection with Pathogen

An estimate of the burden of foodborne disease for New Zealand (Cressey and Lake, 2007) includes an estimate for foodborne salmonellosis of 111 disability adjusted life years (DALYs). This represents 60.7% of the total 186 DALYs for salmonellosis, with the percentage

foodborne being derived from an expert consultation process. This placed foodborne salmonellosis fourth on the list for foodborne disease burden (after campylobacteriosis, norovirus infection, and perinatal listeriosis).

This burden of disease estimate has been supplemented with a cost of illness estimate, based on the same incidence data (Cressey and Lake, 2008). The costs included were direct and indirect medical costs, as well as the value of lost production. This estimated the total cost for salmonellosis as \$4.8 million, with foodborne infections costing \$2.8 million. A more recent report estimated the cost of foodborne salmonellosis as \$15.41 million (Gadiel and Abelson, 2010). This value included a monetisation of the burden of disease on individuals, previously measured as DALYs.

The New Zealand estimates of the burden of foodborne disease from salmonellosis do not subdivide the burden according to specific foods. As part of the cost-benefit analysis accompanying the FSANZ Standard 4.2.5 recently gazetted in Australia<sup>13</sup>, it was estimated that there are about 12,800 cases of egg-related salmonellosis per year in Australia (out of approximately 81,000 foodborne cases per year (Hall, 2004)), costing \$44mAU, and that the number of cases is rising. The cost estimate includes approximately \$6mAU to industry (reputation damage, inefficiencies, product recall), approximately \$2mAU costs to government (compliance and investigation, recalls), and approximately \$36mAU costs to the community (health related costs, loss of income and/or leisure, and monetary value attributed to pain and suffering).

A recent report from the US ranked *Salmonella* in eggs as the tenth highest pathogen-food pair in terms of quality adjusted life years (1,878 QALYs) and cost of illness (\$370m US) (Batz *et al.*, 2011). This report estimated that *Salmonella* in eggs was responsible for 11.2% of foodborne salmonellosis, based on outbreak data.

European estimates of the cost of salmonellosis are similar to New Zealand estimates (given population differences), with Kemmeren *et al.* (2006) estimating the cost of salmonellosis in the Netherlands to be 8.8 million Euros in 2004 (Kemmeren *et al.*, 2006). A cost-benefit analysis of *Salmonella* control in the Danish table-egg sector found that the proportion of egg associated salmonellosis was 55-65% in 1997 compared to 5-7% in 2006 (Korsgaard *et al.*, 2009). The intervention efforts were considered a good investment from a societal (health care and lost productivity savings) point of view.

### 3.6 Adverse Health Effects Summary

The incidence of reported salmonellosis has been stable in New Zealand since at least 2005. The rate of 25-35 reported cases per 100,000 population is close to other developed countries, particularly in Europe, and lower than in Australia. There have been six salmonellosis outbreaks where eggs were a suspected food vehicle, while for two further outbreaks, stronger evidence for eggs as a vehicle was found during investigations. However, for neither of these outbreaks was *Salmonella* detected in raw eggs themselves, which means that it is possible that contamination occurred by cross contamination from another food or an infected food handler. The number of reported domestically acquired infections with *S. Enteritidis* PT4, the type of

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<sup>13</sup> <http://www.foodstandards.gov.au/srcfiles/P301%20Eggs%20PPPS%20DAR%20SD6%20Cost-Benefit.pdf>  
accessed 1 July 2011

*Salmonella* most often associated with trans-ovarian transmission in eggs, is very low, which suggests that this type is not a public health concern in New Zealand at this time.

## 4 EVALUATION OF RISK

### 4.1 Existing Risk Assessments

Prior to the finalisation of the FSANZ Standard for Eggs and Egg Products, a risk assessment was conducted.<sup>14</sup> The quantitative risk assessment for *Salmonella* in eggs by FSANZ was largely drawn from a quantitative model for non *S. Enteritidis* serotypes in eggs developed for the Australian Egg Corporation Ltd. (AECL) (Thomas *et al.*, 2006). A copy of this model was not able to be obtained by the authors of this Risk Profile, although a Risk Profile written by the South Australian Research and Development Institute (SARDI) for AECL is available (Daughtry *et al.*, 2005). The Risk Profile concluded that *Salmonella* is the principal microorganism of human health concern associated with eggs and egg products.

The FSANZ risk assessment addressed both chemical and microbiological hazards, and also concluded that *Salmonella* is the principal microorganism of human health concern associated with eggs and egg products. The risk assessment considered *Salmonella* spp. in eggs from the point of lay through to consumption and was developed to predict the effect of time and temperature of storage on the risk of illness. Results for the quantitative model as well as epidemiological evidence demonstrated that the consumption of uncooked or lightly cooked foods containing raw egg represent risk for foodborne illness.

The FAO and WHO have jointly carried out a quantitative risk assessment (QRA) of *Salmonella* spp. in eggs and broiler chickens, with the section on eggs focussed on *S. Enteritidis* (FAO/WHO, 2002). A risk assessment for *S. Enteritidis* in eggs published by the United States (US) Department of Agriculture's Food Safety and Inspection Service (FSIS) and the Food and Drug Administration (FDA) in 1998 was updated and republished in 2006 (Schroeder *et al.*, 2006). The European Food Safety Authority (EFSA) reported a quantitative risk assessment of *S. Enteritidis* in shell eggs in Europe (EFSA, 2010).

As these risk assessments all focussed on *S. Enteritidis* in eggs and the available information suggests that *S. Enteritidis* is not prevalent in New Zealand layer flocks, the results may not be directly applicable in New Zealand. Interpretation and extrapolation of the results from these risk assessment must be done with caution as they are sensitive to the data used which are most likely biased due to selection effects. If data within the risk assessments are changed to reflect a specific national situation then the impact of an intervention measure would likely change (EFSA, 2011).

Further details of these risk assessments are available in Appendix 2.

### 4.2 Estimate of Risk for New Zealand

#### 4.2.1 Risk associated with eggs

The incidence of notified cases of salmonellosis has declined since a peak of 65 per 100,000 population in 2001, and has been stable in New Zealand since 2005 at 25-35 reported cases per 100,000 population. This rate is close to that in other developed countries, particularly that in Europe, and lower than in Australia. Throughout the 1980s and 1990s the rate fluctuated between 37 and 57 per 100,000 population, with no apparent trend.

To date, results from surveys and the typing data available for New Zealand eggs have not identified *S. Enteritidis* strains which are commonly implicated in vertical (trans-ovarian) transmission in eggs in other countries. *S. Enteritidis* in eggs is therefore not believed to be a significant public health concern in New Zealand at this time. The number of reported human cases of *S. Enteritidis* in general is a small proportion of the total (8.2% of all salmonellosis cases 2000-2010), and *S. Enteritidis* PT4 infections (118 cases) represent 0.7% of the total salmonellosis cases from 2000-2010. The yearly number of reported *S. Enteritidis* PT4 cases has declined over the period 2000-2010, and these cases are strongly associated with overseas travel.

Epidemiological evidence from outbreaks and case-control studies linking eggs with illness in New Zealand is sparse. A survey of retail eggs conducted in 2000 alongside an outbreak case-control study found *Salmonella* on the surface of 14% of egg samples (Thornley *et al.* 2002). However, consumption of eggs was not found to be a significant risk factor. This study only examined cases of infection with *S. Typhimurium* DT160, and this serotype was not found in the eggs. Attribution and aetiology studies have not ranked eggs highly as a source of infection in New Zealand.

There have been a number of outbreaks in Australia of salmonellosis associated with eggs. 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. Two Australian surveys of retail and farm sourced eggs have shown a zero prevalence of *Salmonella* in the eggs. One of these surveys found no *Salmonella* on the shells of eggs, while the other found a prevalence of 3.5%.

Surveys of eggs in New Zealand have not found *Salmonella* in the contents, while the most recent survey (2007) found 1.8% of eggs were contaminated externally. The survey conducted in 2000 parallel with the *Salmonella* Typhimurium DT104 case-control study gave a much higher rate of contamination, but sampling represented a small number of brands (six) and only two of these had positive samples. The 1.8% prevalence from the 2007 survey seems the more reliable figure, representing 49 different brands. All positive samples in both surveys came from caged birds, which is the source of the majority of eggs in New Zealand.

Periodic spikes of contamination of eggs by *Salmonella*, arising from contaminated inputs such as feed or drinking water, or other environmental sources (rodents, pests etc.), are likely to be the pattern for this food/hazard combination.

A report from a US study commented that even a low rate of contamination can represent a large number of contaminated eggs, given the large numbers produced each year (Braden, 2006). Approximately 1 billion eggs are produced in New Zealand each year

This Risk Profile has been commissioned in order to address the following specific risk management questions:

- What is the public health risk from *Salmonella* in and on eggs consumed in New Zealand?
- Has the risk of salmonellosis from consumption of eggs changed since the 2004 Risk Profile?

The risk of salmonellosis from consumption of eggs does not appear to have changed since the 2004 Risk Profile, based on:

- the static incidence of reported illness, which is almost the same as in 2004;
- data supporting the continued dominance of *S. Typhimurium* types in reported cases, rather than *S. Enteritidis*, which is associated with trans-ovarian transmission.

The 2004 Risk Profile commented that there was little evidence that transmission of *Salmonella* via eggs is a significant transmission route occurring in New Zealand. However, the results from the 2001 Auckland survey of eggs deserved further investigation, to determine whether the prevalence was still high, and consistent throughout the national egg supply.

Since that Risk Profile, a larger survey in 2007 has provided an estimate of the prevalence of *Salmonella* contamination on the shells of eggs in New Zealand of 1.8%, which is higher than reported for other developed countries, apart from a recent survey in South Australia. The New Zealand survey also did not find *Salmonella* in egg contents.

In domestic and retail food service settings surface contaminated eggs may contaminate foods when the eggs are cracked for use, and cross contamination may occur during pooling of the eggs. Cross contamination may also occur via hands, utensils and surfaces, or directly to the mouth. Contaminated food may be cooked by methods which will reduce the number of bacteria in the foods but may not entirely eliminate the bacteria.

It is possible that *Salmonella* contaminating the surface of eggs may penetrate into the egg and eventually reach the yolk contents and grow (Zhang et al. 2011). If eggs are refrigerated, then the YMT estimates suggest that this would be a rare occurrence. However, there are only very limited data on the storage conditions and times for eggs by New Zealanders. Egg washing procedures may also create opportunities for shell penetration through cuticle damage, cracking or temperature differentials.

A case-control study in Minnesota found that sporadic cases were significantly more likely to have consumed undercooked eggs, or eggs-containing foods during the three days before onset of illness despite no egg-associated outbreaks of salmonellosis being recognised in the region (Hedberg *et al.*, 1993). This situation may apply in New Zealand. Although unlikely to be a dominant vehicle for transmission of salmonellosis in New Zealand, the estimate that eggs represent the cause of up to 10% of infections appears reasonable (Wilson and Baker, 2009). This would equate to a cost of approximately \$1.5 million based on burden figures reported by Gadiel and Abelson, (2010).

#### 4.2.2 Risks associated with other foods

Risk Profiles with *Salmonella* as the hazard have been written for the most commonly suspected food transmission vehicles (other than eggs):

- Poultry (Lake *et al.*, 2004b) (currently being updated)
- Pork and pork products (Gilbert *et al.*, 2010a)
- High lipid foods made from sesame seeds, peanuts, and cocoa beans (Lake *et al.*, 2010)

The updated draft Profile concerning poultry concluded “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 carcasses by the NMD testing programme from 2005 - 2010, it could be argued that the risk has declined.”

The Risk Profile on *Salmonella* in pork concluded that there were insufficient data available to assess the risk to New Zealanders from *Salmonella* in pork. The limited data that were available suggest a low prevalence of contamination, and pork is rarely identified as a vehicle in reported salmonellosis outbreaks.

The review of information concerning high lipid foods considered that contamination of these foods by *Salmonella* was likely to be sporadic, but when contamination did occur the potential for illness would be high, partly because ingestion of cells in high lipid foods protects them from the acid conditions in the stomach. The Profile concluded that such foods represented a minor component of the overall foodborne risk of this illness to New Zealanders.

For eggs, feed is a potential route for introduction of *Salmonella* into layer stock. A Risk Profile addressing *Salmonella* in animal feed (Cressey *et al.*, 2011), found that the fact that the most common *Salmonella* serotype in finished animal feed in New Zealand in recent years (*S. Tennessee*), based on industry data, occurs infrequently amongst human cases suggest that animal feed may not be a major source of human salmonellosis in New Zealand. However, the available information on the *Salmonella* status of feed and feed ingredients in New Zealand as well as the physiological behaviour of *Salmonella* in such matrixes is not sufficiently comprehensive to assess animal feed as a source of human salmonellosis cases.

Based on these assessments of the foods most commonly associated with *Salmonella* transmission, it may be that there is not a 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 or foodborne transmission may be more important.

#### 4.2.3 Risk assessment options

A quantitative risk assessment for *Salmonella* in eggs for New Zealand would require better (and more recent) information on egg consumption, cooking and handling than is currently available. However, such a risk assessment could draw on the considerable amount of work published in Australia in recent years.

A report has been commissioned by the NZFSA to investigate the feasibility of using microbial subtyping approaches for attribution of human salmonellosis. A study has also been designed to undertake phenotyping and genotyping of collections of *Salmonella* isolates originating from humans, cattle, sheep, pigs, chickens (poultry meat and eggs) and wild birds. The distribution of *Salmonella* subtypes among human and animal sources will be analysed using recently developed source attribution models to estimate, with uncertainty, the proportion of human cases attributable to cattle, sheep, pigs, poultry and wild birds in New Zealand.

The source attribution models will incorporate the typing data generated by the study in conjunction with outbreak data, epidemiological data and expert opinion, in order to help identify food safety interventions that would lead to the reduction of *Salmonella* infection in the human population. This is a collaborative project between ESR and mEpiLab, Massey University and will commence in mid-2011 (Dr Eve Pleydell, Massey University, pers. comm.,

July 2011). It is anticipated that these projects will gain further information regarding the distribution of *Salmonella* subtypes in New Zealand and insight to the sources of infection which is currently unclear and is a major gap for risk assessments.

### 4.3 Data gaps

The data gaps identified in this Risk Profile for *Salmonella* in eggs 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.

## 5 AVAILABILITY OF CONTROL MEASURES

### 5.1 Risk Management Strategy

In March 2009, NZFSA released their *Salmonella* Risk Management Strategy 2009-2012. The Strategy aims to achieve a 30% reduction in the reported annual incidence of foodborne salmonellosis after five years. The strategy focuses on non-typhoid *Salmonella* and begins with a primary focus on intelligence gathering from a wide range of food sectors.

The objectives of the *Salmonella* risk management strategy are to:

- Quantify the proportion of foodborne cases attributable to:
  - specific foods
  - animal feeds
  - domestically produced versus imported foods
  - multiresistant and virulent *Salmonella* genotypes associated with foods
- Identify sources of *Salmonella* contamination of specific foods and animal feeds
- Determine the relative value of different interventions throughout the food chain in reducing the risk of salmonellosis
- Make prioritised risk management decisions on appropriate *Salmonella* control measures across the food chain, and according to data availability
- Design and implement an effective monitoring and review programme to support strategic goals.

An updated version of the strategy was published in 2010 that covers 2010-2013<sup>15</sup>. This included a summary of the situation regarding eggs:

“Most of the egg production and packing sector has also been required to have Risk Management Plans (RMPs) from 2003-2004 to control hazards to human health, including *Salmonella*. Three retail egg surveys (South Island in 1994, Auckland in 2001 and Auckland/Christchurch 2007) have shown an absence of internal contamination of eggs by *Salmonellae*. One survey (South Island, 1994) also showed an absence of external contamination of eggs by *Salmonellae*. The Auckland Survey in 2001 found moderate levels of contamination on egg shell (14% of samples). In the 2007 investigation, 1.8% had shell contamination; all of which were *Salmonella* *Infantis*. Most isolates from eggs are not those associated with human illness.”

### 5.2 Relevant Food Controls

Commercial production of eggs is by one of two systems, conventional caged production systems and alternative production systems, including free range and barn systems. Both systems must adhere to the Animal Welfare (Layer Hen) Code of Welfare 2005<sup>16</sup> as produced by MAF Biosecurity. This Code of Welfare outlines minimum standards for hatchery management, food and water supply, housing, equipment, cage and non-cage systems, stocking density (cage, free range and barn systems), lighting, beak trimming, moult inducement,

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<sup>15</sup> Available at <http://www.foodsafety.govt.nz/industry/general/foodborne-illness/salmonella/strategy.htm> (accessed 26 July 2011)

<sup>16</sup> <http://www.biosecurity.govt.nz/files/regs/animal-welfare/req/codes/layer-hens/layer-hens-code-of-welfare.pdf> accessed 9 May 2011

identification, ventilation, temperature, litter management, disease and injury control, humane destruction and stockmanship.

Under the Animal Products Act 1999, since 2005 most egg producers in New Zealand have been required to operate under a RMP, and information is available from a dedicated web page of the NZFSA (now part of MAF)<sup>17</sup>. Exceptions are made for those producers who meet all of three requirements: have no more than 100 female birds, and sell directly to the consumer, and do not sell to an intermediary of any kind (e.g. a cafe, shop or other third party). A template RMP has been developed by the NZFSA<sup>18</sup>. This template contains agreed storage and shelf-life options based upon then current industry code of practice (Egg Producers, 2002)<sup>19</sup> and on the findings of a range of scientific studies. The template includes specific consideration of controls for *Salmonella* during feed manufacture, as well as vaccination of incoming layer birds. Where eggs are washed, it includes recommendations for egg washing as follows:

- Only fresh, intact eggs that have been ideally cooled to 10-14°C should be washed. This helps to achieve the desired temperature differential between the egg and the wash water.
- Washing should take place as soon as possible after collection as washing will not remove bacteria that have already had time to penetrate the egg.
- Jets of wash water and/or brushes should have complete access to each egg.
- The washing temperature should be 40-42°C (higher may risk cuticle damage).
- Wash water should be purified or filtered to remove organic matter and the microbes.
- The detergent used should be alkaline (capable of raising the pH of the wash water to 10- 11) as acid detergents attack the shell.
- Detergent should be low foaming and improve the dirt removing efficiency of the water.
- A final rinse with clean water containing a sanitiser should be applied, e.g. 100-200 ppm of chlorine, quaternary ammonium compounds or calcium hypochlorite, or 12-25 ppm iodine. A potable water final rinse is required when iodine is used. Iodophors or chlorine-bromine compounds have also been found to be effective. The temperature of the rinse water should always be slightly higher than the wash water, e.g. 43-45°C.
- If the washing machine recirculates the hot, detergent/sanitiser-treated water then care should be taken to ensure that the organic and microbial loading does not increase to unacceptable levels. This is usually done through filtration and periodic water changes (at least daily and more frequently if required). (This is to prevent the build up of organic matter and microbes, including pathogens that can re-contaminate the washed eggs).
- Immediately after washing is completed the eggs should be dried quickly and completely to reduce the risk of any remaining bacteria being aspirated into the egg.
- Drying should be followed by candling where any cracked eggs must be removed.
- Some countries permit the use of mineral oil (paraffin oil) sprays to protect the egg from water loss and the associated increase in air cell volume during cold storage. This protects the egg to some extent from bacterial penetration. Some other coatings have also been trialled successfully, e.g. alginates, polymethacrylic acid, corn promaline, polyvinylidene chloride, hydrolysed sugar derivative.

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<sup>17</sup> <http://www.foodsafety.govt.nz/industry/sectors/poultry-eggs/eggs/> accessed 16 May 2011.

<sup>18</sup> <http://www.foodsafety.govt.nz/elibrary/industry/template-eggs/index.htm> accessed 16 May 2011

<sup>19</sup> <http://www.foodsafety.govt.nz/elibrary/industry/code-practice-egg-cop/egg-cop.pdf> accessed 29 June 2011

The RMP template also specifies temperature limits for egg storage. In general, it recommends that cracked eggs are stored at or below 6°C, while other eggs are stored at or below 15°C (within 2 hours of collection). Cracked eggs are sent for further processing, such as pasteurisation or equivalent treatment for animal consumption. It also recommends eggs for retail sale be held at 15°C with a maximum Best Before date of 35 days from date of lay. However, there are no mandatory requirements for retailers, and most eggs are held at ambient temperatures.

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## 7 APPENDIX 1: HAZARD AND FOOD

The information contained in this Risk Profile is current to the date of publication. Please be aware that new information on the subject may have arisen since the document was finalised.

### 7.1 *Salmonella* spp.

#### 7.1.1 Typing methods

##### 7.1.1.1 *Serotyping*

*Salmonella* serotypes are identified by observing the agglutination of a suite of *Salmonella*-specific antibodies with antigens on the bacterial surface. This is known as the Kauffmann-White scheme. The antigenic formulae of *Salmonella* serotypes are defined and maintained by the World Health Organization (WHO) Collaborating Centre for Reference and Research on *Salmonella*, at the Pasteur Institute in Paris (Brenner *et al.*, 2000; Grimont and Weill, 2007).

Somatic (O) antigens are present on the external surface of the bacterial outer membrane (D'Aoust and Maurer, 2007). The O-antigens can be described as smooth (S), where they are well developed and readily agglutinate with specific antibodies, or rough (R) if the antigens are incomplete and exhibit weak or no agglutination with the O-antibodies.

The flagellar (H) antigen is associated with the flagellin, which is a major component of the flagellar (EFSA Panel on Biological Hazards (BIOHAZ), 2010). *Salmonella* strains can have the ability to express two different compositions of the flagellar antigen, called phase 1 and phase 2, and these strains are described as diphasic (sometimes biphasic). Others only produce one composition (monophasic), and variants producing three (triphasic) or more compositions have been identified.

The Vi antigen is the only capsular (K) antigen detected in *Salmonella* serology, and is only produced by *S. Typhi*, *S. Paratyphi C* and *S. Dublin* (EFSA Panel on Biological Hazards (BIOHAZ), 2010).

The serology is expressed as an alphanumeric code that reads as: O-antigens: H-antigens of first phase: H-antigens of second phase (EFSA Panel on Biological Hazards (BIOHAZ), 2010). As an example, *S. Typhimurium* is denoted 1,4,[5],12:i:1,2. The O-antigens are 1, 4, 5 and 12. Both O-1 and O-5 may be present or absent in strains; underlining means that the factor was determined by a method called phage conversion and square brackets means that the antigen may be present or absent without any relation to phage conversion. The 'i' is a phase 1 H-antigen, and H-1 and H-2 are phase 2 H-antigens. A hyphen is used to indicate that an antigen is absent, for example several *S. Typhimurium*-like strains have been described which lack some of the H-antigens, e.g. 1,4,[5],12:i:- or 1,4,[5],12:-:1,2 (EFSA Panel on Biological Hazards (BIOHAZ), 2010).

The antigenic formula of some *Salmonella* serotypes that have been commonly isolated in New Zealand are as follows (Grimont and Weill, 2007):

- Enteritidis      1,9,12:g,m:-
- Brandenburg    4,[5]12:l,v:en,z<sub>15</sub>
- Infantis        6,7,14:r:1,5

- Saintpaul            1,4,[5],12:e,h:1,2
- Heidelberg        1,4,[5],12:r:1,2
- Virchow            6,7,14:r:1,2

#### 7.1.1.2 Phage typing

Once the serotype is identified, a *Salmonella* isolate can be further subtyped by measuring susceptibility to a panel of bacteriophages. Separate bacteriophage panels have been developed for different serotypes, and the ESR Enteric Reference Laboratory (ERL) in New Zealand routinely determines the phage types of any *S. Typhimurium*, *S. Enteritidis* or *S. Typhi* isolates they receive. The *S. Typhimurium* phage typing method involves testing the ability of 29 bacteriophages to lyse an isolate and is able to distinguish 235 phage types (Anderson *et al.*, 1977; Callow, 1959). Phage typing for *S. Enteritidis* uses 10 different bacteriophages (Ward *et al.*, 1987), and 33 bacteriophages are used to phage type *S. Typhi* isolates (Anderson and Williams, 1956).

#### 7.1.1.3 Molecular methods

Pulsed-field gel electrophoresis (PFGE) is a common molecular method that is able to further distinguish *Salmonella* spp. In New Zealand this technique is usually only applied during cluster or outbreak investigations where it is used to determine whether salmonellosis cases had become ill with the same strain of *Salmonella* and to help link these cases with a source of infection. If PFGE does not adequately discriminate *S. Typhimurium*, another molecular-based test called multiple-locus variable-number tandem repeat analysis (MLVA) can be used.

#### 7.1.1.4 Antimicrobial susceptibility

New Zealand hospital and community laboratories are requested to refer all *Salmonella* isolates from human salmonellosis cases to ESR for typing. ESR also receives *Salmonella* isolates from other sources, including food, animal and environmental sources. Approximately 20% of the non-typhoidal *Salmonella* isolates received by ESR are tested for antimicrobial susceptibility, along with all *S. Typhimurium* phage types that are internationally recognised as being multiresistant. These include *S. Typhimurium* phage types DT104, U302, DT12, DT120 and DT193 (ESR, 2010b).

ESR tests susceptibility to 12 antimicrobials: Ampicillin, cephalothin, chloramphenicol, ciprofloxacin, co-amoxiclav, co-trimoxazole, gentamicin, nalidixic acid, streptomycin, sulphonamides, tetracycline and trimethoprim. All cephalothin-resistant isolates are further tested for the production of extended-spectrum  $\beta$ -lactamase (ESBL) and plasmid-mediated AmpC  $\beta$ -lactamase (ESR, 2010). The 2005-2009 results from this antimicrobial testing programme are summarised in Section 8.1.4.

### 7.1.2 Growth and survival

The following information is taken from a number of different sources but, unless otherwise referenced, is primarily derived from a data sheet prepared by ESR under a contract for the Ministry of Health in 2000-2001. The data sheets are located on the MAF website.<sup>20</sup> They are

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<sup>20</sup> See <http://www.foodsafety.govt.nz/science/other-documents/data-sheets/> (accessed January 2011). ESR originally prepared the data sheets for the Ministry of Health in 2001.

intended for use by regional public health units and will be updated from time to time. Please be aware that new information on the subject may have arisen since this document was finalised.

#### 7.1.2.1 Growth

Temperature: Minimum 7°C, growth greatly reduced at <15°C. Maximum 49.5°C. Optimum 35-37°C. Some evidence for growth at temperatures <7°C exists, but this is serotype specific, the data are still not universally accepted and doubts surrounding the experimentation exist.

pH: Minimum 3.8, optimum, 7-7.5, maximum 9.5. The minimum pH is influenced by other factors such as temperature, acid present, and the presence of nitrite etc.

Atmosphere: Can grow in the presence or absence of air as a facultative anaerobe. The growth rate on beef muscle stored at 20°C under nitrogen is only slightly less than that obtained when stored under air (Grau, 1983). At high concentrations of CO<sub>2</sub> (50-60%), growth is strongly inhibited on beef steak and minced beef at 10-11°C, but at 20°C there is little inhibition (Luiten *et al.*, 1982; Silliker and Wolfe, 1980).

Water activity: Minimum 0.94, optimum 0.99, maximum >0.99.

#### 7.1.2.2 Survival

*Salmonella* are known to survive well in foods, particularly those with low water activity (e.g. flour), and on surfaces.

Temperature: *Salmonella* can survive well in foods for long periods at refrigeration temperatures. In frozen foods, although *Salmonella* numbers are considerably reduced, some survive for long periods. Some foods, including meat, ice-cream and butter, appear to be protective of *Salmonella* during freezing and frozen storage. Rapid freezing promotes survival with lower frozen storage temperatures and less fluctuation giving greater survival (Jay *et al.*, 2003).

Frozen storage temperatures near 0°C result in greater death or injury to bacterial cells. In minced chicken breast (pH 5.8), 60-83% of *Salmonella* cells survived storage at -20°C for 126 days, whereas at -2°C and -5°C only 1.3% to 5.8% of cells respectively were still viable after 5 days (Jay *et al.*, 2003).

pH: *Salmonella* appear to be significantly less tolerant of low pH (pH 2.5; hydrochloric acid) than *Shigella* spp. or *Escherichia coli*. These last two organisms possess additional acid survival systems that are not present in salmonellae (Gorden and Small, 1993; Lin *et al.*, 1995).

Water Activity: Survival in dry environments is a characteristic of these organisms. For example, they can survive in bitter chocolate (a<sub>w</sub> 0.3-0.5) for months. Exposure to low a<sub>w</sub> environments can greatly increase the heat resistance of these organisms.

#### 7.1.3 Inactivation

Note that in microbiological terms “D” refers to a 90% (a decimal or 1 log<sub>10</sub> cycle) reduction in the number of organisms.

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

**D times with heat treatment:** At 60°C usually 2-6 min; at 70°C usually 1 min or less. Some rare serotypes (e.g. *S. Senftenberg*) are significantly more heat resistant than the others, but this organism is not considered to be important as a food pathogen (Doyle and Mazzotta, 2000).

**D times for *Salmonella* spp. can depend on the type of food involved.** Long D times have been reported for experiments with *Salmonella* Typhimurium in milk chocolate. Values reported were up to 1,050 min at 70°C, 222 min at 80°C and 78 min at 90°C (Goepfert and Biggie, 1968).

**pH:** At pH values prohibiting growth, the rate of death depends on the pH, acidulant and temperature (the primary factor).

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

**Radiation:** The effect of gamma or beta radiation on *Salmonella* Typhimurium DT104 in ground pork has been researched (Rajkowski *et al.*, 2006). A mixture of six strains was used to inoculate three ground pork products (of varying fat content). The amount of beta radiation to achieve a 90% reduction was around 0.43 kGy regardless of fat content.

**Disinfectants:** A number of disinfectants have been shown to reduce the prevalence or concentration of *Salmonella* on poultry, see Section 7.2.2.

## 7.2 The Food Supply

### 7.2.1 Egg collection, handling and washing

Egg collection on small farms is often by hand and should be at least daily, and as often as every four hours is ideal. Usually caged hen eggs in larger establishments have semi-automated collection (eggs roll by gravity from cage to collection trough). This type of system has been reported to produce lower contamination rates than eggs laid into nests and the shorter the time between the egg being laid and collected, the lower the contamination of the shell, even under unfavourable conditions (ICMSF, 1998). The eggs are then transferred, usually by hand, to paper or polystyrene trays to be candled and graded. Eggs are stored blunt end up, this prevents the yolk from drifting towards the inner membrane, bypassing the protective barriers in the egg white and possible contamination from any microorganisms that have penetrated to the membrane.

A multi-country European study of *Salmonella* infections in laying hens found that housing in conventional battery cages was a risk factor, compared to floor raised, free range and organic systems (Van Hoorebeke *et al.*, 2010). This was possibly due to the larger flock size in battery systems. Other risk factors were the absence of dry cleaning in between production rounds, and winter season sampling. Significant residual contamination, despite cleaning and disinfection, has been demonstrated on layer farms in the UK (Carrique-Mas *et al.*, 2008).

Translucency may occur in some eggs, which is where lighter colour spots are observed when an egg is candled over a light source. There is some evidence that translucency increases the probability that bacteria will penetrate the egg, although this study was not conclusive as pre-treatment involving washing in ethanol may have damaged the cuticle (Chousalkar *et al.*, 2010).

Widespread prevalence of *S. Enteritidis* on equipment and surfaces in egg packing areas on farms where flocks were infected with this bacterium has been demonstrated (Davies and Breslin, 2003). Cleaning and disinfection processes reduced the prevalence, but did not eliminate contamination. When previously sterilised eggs were passed through the packing house processes, 0.3% of egg passages were found to be contaminated.

The following factors related to washing affect microbial penetration and spoilage (derived from (Stadelman, 1994)) are listed in the Technical Annex of the Egg Producers Federation of New Zealand Code of Practice (Egg Producers, 2002):

- Washing eggs in liquid that is at a lower temperature than the eggs results in liquid (plus any bacteria in it) being drawn through the pores. The temperature of the liquid should be at least 12°C higher than the temperature of the eggs.
- Visibly dirty eggs tend to have a higher spoilage rate than those that are clean.
- Any process that wets the shell increases spoilage.
- Damage to the cuticle results in increased microbial penetration.
- Wash water containing iron increases the iron level in the albumen, neutralising the antimicrobial effect of conalbumin. Wash water should have less than 2ppm Fe(III). Levels above 5ppm may greatly accelerate spoilage and growth of pathogens.
- The use of potable water, disinfectants or alkaline detergents reduces (*sic*) the microbiological impact of washing.

When eggs are immersed in a bacterial suspension of a lower temperature than the internal egg temperature, a pressure gradient is set up and bacteria may be drawn in through the shell (Moats, 1978). Freshly laid eggs have a temperature of 41°C (Baker, 1990).

The pH of the washwater has been shown to be important for the survival of *S. Enteritidis* and the ability of the organism to cross contaminate from inoculated to uninoculated shell egg surfaces during immersion washing (Catalano and Knabel, 1994). Washwater at pH 11 and 37.7°C was shown to prevent cross contamination and reduce the level of contamination on eggs inoculated prior to washing, due to reduced survival of the bacteria at this high pH. Slow chilling of eggs after washing at pH 9 from 37.7°C down to 7.2°C over 2-3 days permitted greater survival on the shell, and penetration into eggs. Rapid chilling over 2-4 h prevented internal contamination.

The nature of chemicals used for washing eggs has been reported to influence the egg's subsequent susceptibility to bacterial penetration by *S. Enteritidis* (Wang and Slavik, 1998). The effect of washing eggs with three different commercial egg-washing chemicals has been studied. A quaternary ammonium compound (pH 7.5) and sodium hypochlorite (pH 7.5) both reduced bacterial penetration (compared to water control), while sodium carbonate (pH 12) facilitated penetration. This was attributed to alterations in the egg shell surface. Different storage temperatures (4°C or 23°C) did not result in significantly different penetration rates for up to 21 days. Note that these results are at variance with the recommendation that alkaline solutions are best at reducing bacterial survival (See Section 7.2).

Studies into the efficacy of egg cleaning compounds (sodium carbonate, sodium hypochlorite, potassium hydroxide) tested the ability of the compounds to reduce numbers of *S. Enteritidis* on eggshells after dipping them into solutions containing 2, 4 and 6 log<sub>10</sub> cfu/ml (Soljour *et al.*, 2004). The treatment solutions were applied by immersing the inoculated and dried eggs in pH adjusted solutions at 48°C for 10 minutes. At the recommended concentrations the solutions did not completely eliminate *Salmonella*, although reductions were greater when the pH was 12 than pH 10 or 11.

Best practice guidelines for machine spray washing of eggs under commercial conditions have been published (Hutchison *et al.*, 2004). This study investigated the effect of different conditions and chemicals on eggs externally inoculated with *S. Typhimurium* and *S. Enteritidis*. The inoculation was conducted so that freshly laid eggs were exposed before the cuticle had oxidised/hardened. Reductions in the number of external *Salmonella* of 5-6 log<sub>10</sub> cfu per egg could be achieved. Provided the temperatures of wash and rinse waters were 44°C and 48°C respectively, no internal contamination of the eggs was detected. Lower temperatures resulted in residual contamination within some of the eggs. Other factors such as wash chemical concentration (Clorwash or Quat), the length of washing time, lowered jet pressure and age of the laying bird did not appear to influence contamination of the contents.

Studies have found that other chemical sanitising dips or sprays (particularly dilute hydrogen peroxide) can be beneficial in reducing *Salmonella* contamination of the exterior shell (reviewed in Berrang *et al.*, 2000). Ultraviolet light has also been studied as a means of (non-contact) egg shell sterilisation, and for decontamination of egg handling facilities (Gao *et al.*, 1997; Kuo *et al.*, 1997). Dipping eggs in boiling water for 3-5 seconds is effective in sterilising the exterior of eggs carrying large numbers of *Salmonella* (7.5 log<sub>10</sub> cfu in the shell and membranes) but resulting in some cracking of the shell (Himathongkham *et al.*, 1999). Hot air pasteurisation (2 x 8 seconds of exposure to air at 600°C) of externally inoculated eggs was effective at reducing the load of *S. Enteritidis*, but only by approximately 1-2 log<sub>10</sub> cfu (Manfreda *et al.*, 2010; Pasquali *et al.*, 2010)

#### 7.2.1.1 Contamination of eggs contents through the shell (trans-shell, horizontal transmission)

The survival of *S. Enteritidis* on the shell and membrane at 20°C has been shown to be dependent on relative humidity (RH), with increased survival at higher RH values (Himathongkham *et al.*, 1999). It has been speculated that better survival at low temperatures is due to slower metabolism on dry eggshell surfaces (Messens *et al.*, 2005). Although higher RH and lower temperatures are supposed to enhance survival on shells, *S. Enteritidis* inoculated in broth onto shells was detected on only a few samples after two weeks post-inoculation storage at 25°C (63% RH), and not at all after 4 and 8 weeks storage (Lublin and Sela, 2008). No samples stored at 6°C (90% RH) were positive. In the same study, *S. Virchow* was not detected on any samples post-storage. These results suggest that in the absence of faecal material, survival of *Salmonella* on shells is generally less than two weeks at room temperature, and less at refrigeration temperatures.

Some published reports have suggested a relationship between eggshell quality and bacterial penetration. Measures of egg shell quality include conductance (a measure of porosity), and shell strength and thickness. The latter is positively correlated with specific gravity. The number of pores per egg (6,000 – 10,000) increases with an ageing flock which gives a lower specific gravity for eggs from older hens (ICMSF, 2005). While decreasing specific gravity of

eggs has been associated in some reports with an increase in ability for *Salmonella* to penetrate shells, an examination of eggs from flocks of various ages found no correlation with specific gravity (Berrang *et al.*, 1998) and depended more on the age of the egg (after lay) and the number of bacteria on the shell surface (ICMSF, 2005). Consequently, factors other than shell quality were considered to be important for the penetration of *Salmonella* through the shell. Another study found no correlation between bird age and penetration by *Salmonella* into eggs (Hutchison *et al.*, 2004).

Shell strength is influenced by two factors:

- the hen's diet, particularly its calcium, phosphorus, manganese and vitamin D intake (Lichovnikova, 2007);
- the egg size, which increases as the hen ages while the mass of shell material that covers it stays fixed. Hence the shell is thinner on larger eggs (Rodriguez-Navarro *et al.*, 2002)

Experiments on the penetration of *Salmonella* into eggs have been conducted by contacting inoculated chicken faeces onto the shell surface, incubating the assembly for 30 minutes at up to 35°C, and then further incubation at 4 or 25°C (Schoeni *et al.*, 1995). The presence of *Salmonella* on the inner shell, membrane just under the shell, and contents were determined. *S. Enteritidis*, *S. Typhimurium*, and *S. Heidelberg* penetrated all the way into the egg contents by day 3 at 25°C, while penetration was reduced at 4°C. The greatest penetration was observed when a temperature differential was created between the initial incubation temperature, and the storage temperature e.g. incubation at 35°C for 30 minutes followed by storage at 4°C. This scenario mimics hatchery conditions and emphasises the importance of rapid removal of any faecal contamination. Penetration was also increased by a higher initial inoculum level. In the inoculated experimental faeces, growth of *Salmonella* was observed at 25°C.

Experiments into the penetration of egg shell membrane by *S. Typhimurium* found that the time required for penetration increased with the age of the hen (Berrang *et al.*, 1999). This did not appear to be correlated with the relative open interfibre area of the membrane, although this decreased with hen age. It was speculated that charge or chemical structure may be more important. In contrast, other studies have found that the amount of penetration increased slightly with the age of the hen (30-80 weeks), but this was not statistically significant (De Reu *et al.*, 2006). Of the other egg shell characteristics studied, the only one that was correlated with penetration was the amount of cuticle deposition (measured using a staining technique). As most penetration occurred in the first two days after laying, it appears that cuticle quality is a key factor.

The age of the egg has been shown to influence the degree of penetration by *S. Enteritidis* and *S. Typhimurium* (Miyamoto *et al.*, 1998), with eggs tested immediately after laying (0.25-3h) being more susceptible to penetration than older eggs. Penetration was concentration dependent (suspensions of 6 log<sub>10</sub> cfu/ml caused penetration more frequently (95-100%) than suspensions at 3 log<sub>10</sub> cfu (75-90%)). Penetration could be reduced by cooling eggs at 4°C for 15 minutes prior to exposure to the pathogen. The susceptibility of eggs to penetration immediately after laying (1-3 minutes old) has also been shown for bacteria in general (Sparks and Board, 1985).

While the cooling of eggs after lay is desirable to reduce the potential for penetration and subsequent growth of *Salmonella*, overly rapid cooling, and/or cooling to too low a temperature, may enhance the potential for penetration. Studies involving rapid cooling of eggs to 0°C in convection chambers (time taken: 2 hours with forced air convection, 4.5 hours

with natural convection) found that penetration of *S. Enteritidis* was highest in eggs cooled with forced air (100%) compared to natural convection (>90%), which was higher than control eggs (no cooling, 43% penetration) (Fajardo *et al.*, 1995). Although visible cracks were not found on the eggs, electron microscopy revealed increased numbers and width of microscopic cracks on the cooled eggs. The high rate of penetration in the control group of eggs was probably due to the fact that the eggs came from 72 week old hens, which were chosen because older hens lay bigger eggs with thinner shells which are more prone to cracking. In addition the experiments were done under conditions promoting penetration (inoculated dipping solution at 5°C for eggs at room temperature creating a temperature differential).

The reliability of results from penetration experiments have been questioned, in that unless effective sterilisation of the egg exterior is achieved before opening, cross contamination of contents may occur (Himathongkham *et al.*, 1999). These studies found that effective sterilisation of the exterior and an alternate method of opening the egg generated zero prevalence of internal contamination, compared to the standard method which found up to one third of eggs contaminated internally. It was suggested that this problem may also affect egg survey results.

Work has been undertaken in European Union projects SABRE<sup>21</sup> and RESCAPE<sup>22</sup> to investigate the genetics and physiology of the eggs and chickens as well the use of alternative housing regimes during production with the aim of improving egg quality and food safety in eggs. It is anticipated that these projects will result in better methods to detect and quantify bacterial penetration of egg shells, allow better definition of bacterial penetration and may provide recommendations for the control of penetration of microorganisms in eggs. The Australian CRC are also funding projects that are investigating methods to improve the efficacy and safety of egg washing<sup>23</sup> as well determining the effects of defects in shell quality and structure on bacterial penetration into the egg<sup>24</sup>.

#### 7.2.1.2 Growth of *Salmonella* in and on eggs

Bacteria contaminating the outside of shells are unlikely to grow as nutrients and moisture levels would be too low. *Salmonella* survive on cleaned eggs for only a few days and internal contamination on a clean, uncracked fresh shell egg is rare. It has been suggested that survival of *Salmonella* on the shells of eggs is longer at lower temperatures due to the slower metabolism induced by the disadvantageous conditions on the dry eggshell surface (Radkowski, 2002, Baker, 1990). Nevertheless, refrigerated storage is desirable to inhibit the penetration of bacteria into the egg and inhibit the growth of *Salmonella* that do penetrate into the egg contents.

A number of studies have indicated that *S. Enteritidis* can survive in the egg content but it is unclear whether it can grow in the albumen as many studies show conflicting results. These differences between studies may be due to various factors in the experimental methodology used including different inoculum size, strains, incubation temperatures, storage times and age of eggs (Zhang *et al.* 2011). One study had reported that *S. Enteritidis* growth was less frequent

<sup>21</sup> [http://ec.europa.eu/research/agriculture/success\\_sabre.en.html](http://ec.europa.eu/research/agriculture/success_sabre.en.html). Accessed 16 November 2011

<sup>22</sup> [http://ec.europa.eu/research/biosociety/food\\_quality/projects/161\\_en.html](http://ec.europa.eu/research/biosociety/food_quality/projects/161_en.html). Accessed 16 November 2011.

<sup>23</sup> <http://www.poultryhub.org/research/researchto-improve-the-efficacy-and-safety-of-egg-washing/>. Accessed 17 November 2011.

<sup>24</sup> <http://www.poultryhub.org/research/researcheggshell-quality-and-risks-of-food-borne-pathogens/>. Accessed 17 November 2011.

at lower inoculum doses (15 cells), shorter storage times (1 day) and lower temperatures (10 – 17.5°C) (Gast and Holt 2000). The microorganism can also grow in the contents of naturally contaminated eggs in room temperature and multiplication of *Salmonella* in an egg could occur rapidly within a single day of storage at a warm temperature. Inoculation experiments found that *S. Enteritidis* reached a mean level of 8.4 – 8.7 log units/ml at 2 d in yolk samples initially contaminated with 15 and 150 cfu respectively and up to 6.1 log units/ml (150 cfu dose) in whole egg at day 2 (Gast and Holt 2000).

Growth of *Salmonella Enteritidis* is rapid in egg yolk at 25°C and slows, as would be expected, with decreasing temperature, with minimal growth occurring at 10°C (Gast and Holt, 2000; Humphrey *et al.*, 1989). High numbers (around 8 log<sub>10</sub> cfu/ml) were reached in yolk after 2 days incubation at 25°C, from an inoculum of only 15 cfu. There was almost no growth in albumen and whole egg (inoculated at the albumen edge) at this temperature, while at the yolk surface growth reached approximately 5 log<sub>10</sub> cfu/ml after 3 days. At 10°C growth occurred only in yolk, and was limited, reaching 3 log<sub>10</sub> cfu/ml after 3 days (Gast and Holt, 2000).

Growth in the albumen is slower; *S. Enteritidis* increased by only 2 log<sub>10</sub> cfu in egg white at 30°C over 4-6 days, which was attributed to inhibition from iron binding by ovotransferrin (Baron *et al.*, 1997). *Salmonella Typhimurium* has been shown to grow in whole and blended eggs at 12°C, but not at 7°C when incubated for 24h (Baker *et al.*, 1983). At 37°C, *S. Enteritidis* gradually declined in egg albumen, but persisted in the yolk (Baker, 1990).

Different isolates have been shown to reach different maximum numbers in liquid whole egg incubated at 37°C (Gast and Holt, 1995). Twelve isolates were tested and the final numbers attained varied by 3.5 log<sub>10</sub> units, with some reaching numbers in excess of 7.5 log<sub>10</sub>/g. This effect was largely abolished by the addition of iron and so presumably reflects differences among the isolates in their ability to sequester iron. Further exploration of serotype variability in growth potential has shown the importance of motility elements (flagella and curli fimbriae) in facilitating growth in egg contents (Cogan *et al.*, 2004). This was attributed to the requirement for movement of bacteria through the albumen towards the yolk. Most of these experiments were done with strains of *S. Enteritidis* but two strains of *S. Typhimurium* (one was DT104) also showed a high level of yolk invasion. These two strains were also able to grow to high numbers (>6 log<sub>10</sub> cfu/ml) in approximately 25% of inoculated eggs stored for 8 days at 20°C, which was the highest percentage achieved by any of the *S. Enteritidis* strains. This supports the assumption that data on the behaviour of *S. Enteritidis* in eggs can be extrapolated to other serotypes. However, it also points to the possibility that *S. Typhimurium* may be involved in trans-ovarian transmission, given the association between fimbriae and ovarian infection. The ability of eight strains of *S. Typhimurium* DT104 to contaminate the interior of intact eggs laid after infection of chickens (by oral inoculation) has been demonstrated (Williams *et al.*, 1998). Faecal carriage was detected for all strains, although the percentage of infected chickens declined across the two week experimental period. Only one strain was detected in muscle tissue. The eggs were removed “as soon as possible after lay” so although penetration by bacteria from faeces cannot be ruled out, it seems likely that the egg contents were contaminated during development.

Contamination of eggs at 25°C where *S. Enteritidis* was introduced onto the inner membrane of the air cell was determined to be greater when the air cell was uppermost than when it was downwards (Clay and Board, 1991). This appeared to be due to movement of the yolk upwards towards the inoculation site due to density changes over time. Growth of *S. Enteritidis* in both yolk and albumen was rapid, reaching 8-10 log<sub>10</sub> cfu/ml in eggs stored at 25°C after 20 days.

In eggs stored at 4°C or 10°C growth was prevented and inhibited respectively, but eggs stored at 10°C and then transferred to 25°C showed rapid growth.

The growth of *S. Enteritidis* in the yolk of eggs which had their albumen inoculated did not occur in eggs stored for less than three weeks at 20°C (Humphrey and Whitehead, 1993). However, growth has been reported in eggs incubated at 8°C for two of three isolates after only a few days (Baker, 1990) (although most yolks remained negative and the contamination did not seem to increase with incubation period). Growth after this time appeared to be associated with a change in the membrane permitting invasion of the yolk or leakage of nutrients from the yolk into the albumen. Growth of the organism was minimal in albumen separated from the yolk.

*S. Enteritidis* inoculated directly onto the vitelline membrane enclosing the yolk has been shown to grow at high temperatures (37°C, 27°C and 15°C), with an increase of up to 4.5, 2.1 and 1.0 log<sub>10</sub> cfu in 2 days respectively (Fleischman *et al.*, 2003). At lower temperatures (8°C, and 4°C) the numbers of bacteria declined. These experiments also showed no growth or a reduction in numbers for *Salmonella* in albumen at these temperatures, while in yolk rapid growth occurred at the three higher temperatures (5.1, 4.2 and 3.5 log<sub>10</sub> cfu increase respectively). No growth occurred in yolk at 8°C and 4°C.

The number of organisms present initially may be very small, and they are unlikely to grow until such time as they can penetrate the vitelline membrane and contaminate the yolk. Alternatively, membrane breakdown may allow yolk contents to enter the albumen and facilitate growth. The rate of change in membrane permeability is temperature dependent i.e. storage temperature is important, with refrigerated storage (<8°C) after purchase helping to prevent rapid growth. Fluctuations in temperature also appear to increase permeability (Humphrey and Whitehead, 1993). Although there are some data to show that maintaining temperatures below 20°C is sufficient to control growth, this was considered difficult to achieve in retail outlets (Humphrey, 1994). It is also claimed that unlike spoilage organisms, growth of *Salmonella* in egg contents does not affect appearance or odour, until very high numbers are reached (Humphrey, 1994).

The concept of Yolk Mean Time or Yolk Membrane Breakdown Time (YMT) has been developed (FAO/WHO, 2002; Whiting *et al.*, 2000) to describe the number of days at a given temperature before growth of *S. Enteritidis* in the egg begins. This approach has been used in risk assessments by the USDA<sup>25</sup>, FAO/WHO<sup>26</sup> and FSANZ<sup>27</sup> to assess the potential for growth before consumption. Once the time required for yolk membrane breakdown is exceeded, the bacteria grow at a rate described by an exponential equation, and can be very rapid at higher temperatures. Growth is very slow at temperatures below 7°C, but rises to approximately 0.2 log<sub>10</sub> cfu per hour at 23°C (Table 4.16 in the FAO/WHO risk assessment).

Growth of *S. Typhimurium* DT104 as a result of post-pasteurisation contamination of commercial liquid egg products has been assessed (McQuestin *et al.*, 2010). The numbers of bacteria slowly declined at 4°C, but increasing amounts of growth occurred at higher

<sup>25</sup> <http://www.fsis.usda.gov/ophs/risk/contents.htm> accessed 4 July 2011

<sup>26</sup> [http://www.fao.org/ag/agn/agns/jemra\\_riskassessment\\_salmonella\\_en.asp](http://www.fao.org/ag/agn/agns/jemra_riskassessment_salmonella_en.asp) accessed 4 July 2011

<sup>27</sup>

[http://www.foodstandards.gov.au/\\_srcfiles/P301%20Eggs%20PPPS%20DAR%20SD1%20Risk%20Assessment.pdf](http://www.foodstandards.gov.au/_srcfiles/P301%20Eggs%20PPPS%20DAR%20SD1%20Risk%20Assessment.pdf) accessed 4 July 2011

temperatures (10-42°C) in whole egg, albumen, and 10% sugared yolk. No growth was observed in 10% salted yolk, and the numbers of bacteria slowly declined. However, the addition of water activity ( $a_w$ ) lowering salt at 10% has also been shown to increase the thermal resistance of *Salmonella* and this would inhibit the control of residual bacteria by cooking (Palumbo *et al.*, 1995).

### 7.2.2 Pasteurisation and cooking controls for *Salmonella* in eggs

Intact shell eggs inoculated into the yolk with approximately  $8 \log_{10}$  cfu *S. Enteritidis* required thermal treatments of 50-57 minutes at 58°C, or 65-75 minutes at 57°C, to completely eliminate the pathogen (Schuman *et al.*, 1997). These results provide D values of 4.5 and 6.0 minutes for 58°C and 57°C respectively, after the initial 24-35 minute time for the egg interior to reach the temperature of the water. The quality of the albumen was affected but such immersion-pasteurised eggs were still suitable for numerous culinary uses.

Other investigations of whole egg pasteurisation found a  $3 \log_{10}$  reduction when eggs were heated in a circulating water bath operating at 57°C for 25 minutes and a  $5 \log_{10}$  reduction when eggs were exposed to dry heat for 180 minutes at 55°C. A combination of the two, with the dry heat stage reduced to 60 minutes produced a  $7 \log_{10}$  reduction (Hou *et al.*, 1996). This process is claimed to give a  $6 \log_{10}$  margin of safety when considering levels of *S. Enteritidis* normally found in eggs.

D values for a six isolate mixture of salmonellae in liquid egg yolk were 0.57 minutes at 61.1°C, 0.20 minutes at 63.3°C, and <0.20 minutes at 64.4°C (Palumbo *et al.*, 1995). However, the addition of NaCl or sucrose increased the D value (increased thermal resistance). For example the D time at 63.3°C was 11.5 minutes when the yolk contained 10% NaCl. When  $a_w$  lowering solutes were added the thermal death curve showed distinct shoulders and tailing, i.e. was not log linear. The log reductions measured for various egg products pasteurised to standards were as below:

<u>Product</u> <u><i>Salmonella</i></u>	<u>Temp (°C)</u>	<u>Time (min)</u>	<u>Log<sub>10</sub> reduction</u>
Egg yolk	61.1	3.5	6.14
Egg yolk + 10% sucrose	63.3	3.5	4.86
Egg yolk + 10% NaCl	63.3	3.5	0.30
Egg yolk + 10% NaCl + 5% sucrose	63.3	3.5	0.43
Egg yolk + 20% NaCl	64.4	3.5	0.76

In experiments using nine strains of *S. Enteritidis* in whole egg, the D time at 60 minutes varied from 0.69 to 0.31 minutes (Baker, 1990). The mean D time was 0.42 minutes with a standard deviation of 0.1 minute. The US recommendation for pasteurising liquid whole egg of 60°C for 3.5 minutes was found to cause at least a 7D reduction for all but one strain.

Similar experiments have been carried out with liquid egg whites (Palumbo *et al.*, 1996). The pasteurisation of egg white is difficult because of the lack of functionality of the food on heating, i.e. albumen is denatured in a few minutes at or above 60°C. Homogenised whole egg and yolk are reasonably stable at this temperature (ICMSF, 2005). Pasteurisation standards in the US allow for heating regimes where hydrogen peroxide is added to the egg white, with

residual peroxides being removed by the addition of catalase, and the addition of hydrogen peroxide increases the heat sensitivity of salmonellae. D values for a six isolate mix of salmonellae were 3.87 minutes at 51.5°C and 1.60 minutes at 53.5°C in the presence of 0.875% H<sub>2</sub>O<sub>2</sub> in egg white of pH 8.8. In the absence of hydrogen peroxide, D values were 2.74 minutes at 55.5°C, 1.44 minutes at 56.6°C and 0.78 minutes at 57.7°C. The log<sub>10</sub> reduction of *S. Senftenberg* in pasteurisation processes meeting the US standards during plate pasteurisation varied from 3.64 to 1.80. The pH was also an important factor with the log<sub>10</sub> reduction at 56.6°C for three minutes being 3.60 at pH 7.8 and 1.08 at pH 9.3. This is important as the pH of egg white rises from around 8.2 to 8.9-9.1 with time. It was concluded that the current time and temperature combinations do not provide a 99.99% (4D) reduction in salmonellae in commercial egg white.

D times for a number of *Salmonella* isolates heated in liquid egg white and yolk are presented below (Chantarapanont *et al.*, 2000):

Serotype	Phage type	D Value (min)	
		in liquid albumen at 52°C	in liquid yolk at 56°C
Enteritidis	4	5.18	5.85
	4	3.82	6.38
	8	4.49	7.39
	13a	4.57	5.85
	34	3.91	6.16
	34	3.76	5.14
Senftenberg		13.43	19.96

Differences in D values have also been demonstrated for *S. Enteritidis* strain 13076 in liquid whole egg and liquid egg white as shown in Table 6 (Jin *et al.*, 2008). In these studies the D times at 56°C in liquid whole egg were much lower than in liquid yolk above.

**Table 6: D values for *S. Enteritidis* Strain 13076**

D Time (Standard deviation)	52°C	54°C	56°C	58°C	60°C
Liquid whole egg	ND	5.70 (3.15)	0.82 (0.37)	0.27(0.02)	0.17(0.003)
Liquid egg white	6.12 (2.13)	1.51 (0.17)	0.42 (0.07)	0.19 (0.02)	ND

ND Not determined

Experiments with eggs cooked to simulate whole, boiled, fried and scrambled eggs have shown that salmonellae in the yolks can survive while the yolk is still liquid (Humphrey *et al.*, 1989). Whole eggs inoculated with approximately 7 log<sub>10</sub> cfu/g still contained viable organisms in the yolk after 4 minutes boiling (the temperature of the yolk reached around 56°C). Eggs containing the same inoculum fried “sunny side up” still contained countable numbers of organisms, while those cooked “over easy” could still yield the inoculum after enrichment. Different approaches to cooking scrambled eggs gave different inactivation of the inoculum, depending, unsurprisingly, on the final temperature reached. Microwave cooking could be as

efficient as cooking in a pan. At times normally used for boiling eggs, the inoculum size was not correlated with overall survival as long as the yolk was still liquid after cooking.

The kinetics of *S. Enteritidis* destruction in eggs by boiling show that the initial rate of decline is slow, followed by a sudden drop in numbers to zero (Grijnspeerdt and Herman, 2003). This could be expected based on the process of internal temperature increase. These experiments showed that for initial numbers of *Salmonella* of approximately  $8 \log_{10}$  cfu/ml, complete elimination (as judged by the methods used) of the bacteria took between 5 minutes (egg at room temperature placed in boiling water) and 12 minutes (egg at refrigeration temperature added to water at room temperature before heating).

For *S. Typhimurium* inocula of around 4-5  $\log_{10}$  cfu/g were only destroyed when the yolk of a boiled egg reached 75.4°C (Baker *et al.*, 1983). Poaching and the cooking of omelettes were found to destroy the organism, whereas eggs fried “sunny side up” were not free of the inoculum.

In an investigation into hard-cooking methods, eggs inoculated with  $10^7$ - $10^8$  salmonellae inoculated into the yolk were cooked according to two methods (Chantarapanont *et al.*, 2000):

- the American Egg Board method which is to place eggs into water at 23°C, heat to 100°C, remove from the heat and hold for 15 minutes; and,
- placing eggs in water at 100°C, then holding for 15 minutes at that temperature.

As might have been expected, inactivation by these treatments was slower in eggs that were at an initial temperature of 10°C than 21°C, and slower in large eggs than medium eggs. *Salmonella* could be recovered from eggs cooked by the first method for up to 9 minutes, whereas after 9 minutes at the second method, *Salmonella* could still be recovered from extra-large eggs. It was generally recommended that regardless of the method used to hard cook eggs, sufficient time in boiling water should be given to completely solidify the yolk.

In further studies some consideration of time and temperature has been made. The results are summarised below:

<u>Cooking method</u>	<u>Inoculum (cfu/ml)</u>	<u>Time needed for complete kill</u>	<u>Final temperature (°C)</u>
Scrambling	$4.2 \times 10^5$	2 min.	74
Poaching	$3.2 \times 10^4$	5 min.	75
Boiling	$5.9 \times 10^4$	7 min.	75
Frying:	$2.7 \times 10^5$		
Covered		4 min.	70
Sunnyside up		7 min.	64
Turned over		3 + 2 min.	61

When hands were used to crack eggs inoculated with *S. Enteritidis* up to 25% of fingers tested were positive for *Salmonella* and, even after washing, 1.8% of fingers remained positive (Humphrey *et al.*, 1994). When batter mixes were prepared with an electric mixer, *Salmonella* was dispersed up to 40cm from the mixing bowl without any obvious associated splashing of the mix. Most importantly *S. Enteritidis* PT4 survived for 24 h on a soiled formica surface

even when present at low initial numbers. Cross contamination was almost instantaneous when ready-to-eat food was placed onto egg droplets containing this organism (Bradford *et al.*, 1997). Somewhat longer exposure was required for transfer when the egg droplets were allowed to dry.

### 7.2.3 Cooking methods

Heat treatment of eggs and egg-containing foods is highly variable from 'raw' to 'well cooked'. An analysis of four surveys on egg consumption in the US has been published (Lin *et al.*, 1997). In one of these surveys, 27% of egg-containing dishes were described as undercooked, with each person consuming undercooked eggs 20 times a year. The meals involved were eggs fried over easy and sunny side up 49%, scrambled eggs 29%, poached eggs 13%, soft-boiled eggs 7% and hard boiled eggs 2%.

A telephone survey of 1,260 people in the US, determined that 53% of respondents ate raw eggs (Klontz *et al.*, 1995). Examples of raw egg-containing foods were; cookie batter, homemade ice cream, homemade egg nog, Caesar salad, frosting, homemade shakes, homemade hollandaise sauce and homemade mayonnaise. A cross-sectional telephone survey of 2,332 randomly selected residents in the Waterloo region in Ontario, Canada, found that elderly respondents were the most likely group to consume undercooked eggs (Nesbitt *et al.*, 2008).

### 7.2.4 Prevalence of *Salmonella* on eggs overseas

The European Union (EU) has set for the United Kingdom (UK) an annual target of 10% reduction in the prevalence of *S. Enteritidis* and *S. Typhimurium* in commercial egg laying holdings. To provide a baseline estimate for this target, data from commercial egg laying flocks were analysed using Bayesian methods (Arnold *et al.*, 2010). The results indicated the prevalence of infected holdings for all *Salmonella* serovars was 18% (95% credibility interval 12-25%). For *S. Enteritidis* and *S. Typhimurium* only the prevalence was 14% (95% credibility interval 10-20%). These estimates were higher than previous analyses (11.9%), because the uncertainty caused by testing only one flock per holding and test sensitivity were taken into account. This analysis also supported the need for increased monitoring to provide future robust estimates. Such increased monitoring has been introduced under the 2008 UK National Control Plan for *Salmonella* (see Section 9.2).

Studies of the prevalence of *Salmonella* in eggs, egg products and layer hens are summarised in Table 7, Table 8 and Table 9 respectively. The emphasis is on surveys conducted since 2000. One longitudinal study in high rise (multiple level) poultry houses has demonstrated that older layer hens have a lower prevalence of *Salmonella* although the numbers of bacteria in faeces was not correlated with age (Li *et al.*, 2007).

The 2010 report from a survey of commercial eggs in Australia which did not find *Salmonella* on the shell or internal contents of 500 eggs tested (Chousalkar *et al.*, 2010), also referred to an earlier larger survey reported to the Australian Egg Corporation in 2005. In this study *Salmonella* was not isolated from the surface of 10,000 or the internal contents of 20,000 eggs sampled.

A review of the serovars isolated internationally from table eggs has been published recently (Martelli and Davies, 2011).

**Table 7: Prevalence of *Salmonella* in and on shell eggs**

Country (Reported Year)	Investigated Year	Sample	No. of Samples	No. of eggs pooled	Object	% Positive	Serotype information (% of isolates)	Reference
Albania (1998)	1997	Imported eggs	7	5	On egg	14.29%	Group C1 (100.0)	(Telo <i>et al.</i> , 1998)
					In egg	0.00%		
Albania (1999)	1996-97	Imported shell eggs	79	5	On egg	1.27%	Group C1 (100.0)	(Telo <i>et al.</i> , 1999)
					In egg	0.00%		
Australia (2008) (Adelaide)	NS	Retail Eggs	199	1	On egg	3.50%	<i>S. Infantis</i> (29) <i>S. Typhimurium</i> 135 (29) <i>S. Johannesburg</i> (14) <i>S. Typhimurium</i> RDNC (14) <i>S. Livingstone</i> (14)	(Fearley <i>et al.</i> , 2011)
					In egg	0.00%		
Australia (2010)	NS	Eggs from farms	500	1	On egg	0.00%		(Chousalkar <i>et al.</i> , 2010)
					In egg	0.00%		
Canada (1998)	1996	Table eggs	252	6	Whole	0.40%	<i>S. Agona</i> (100.0)	(Poppe <i>et al.</i> , 1998)
Chile (2000)	1998-99	Eggs	1081	12	On egg	0.00%	<i>S. Enteritidis</i> (100.0)	(Alexandre <i>et al.</i> , 2000)
					In egg	0.09% <sup>+</sup>		
China (2007)	2003-05	Raw eggs	58	1	NS	0.00%		(Chao <i>et al.</i> , 2007)
China (2007)	NS	Eggs	25	1	NS	4.00%	<i>S. Enteritidis</i> (100.0) <sup>1</sup>	(Hu <i>et al.</i> , 2007)
Germany (2006)	2004	Eggs entire	10179	NS	Whole	0.44%	<i>S. Enteritidis</i> (89.3) <i>S. Typhimurium</i> (2.4) Other (7.1)	(Hartung, 2006)
		Shells	8968	NS	On egg	0.42%		
		Egg white	1870	NS	In egg	0.00%		
		yolk	9160	NS	In egg	0.02%		
Germany (2007)	2005	Eggs entire	8285	NS	Whole	0.51%	<i>S. Enteritidis</i> (84.7) Other (5.5)	(Hartung, 2007a)
		Shells	6876	NS	On egg	0.41%		
		Egg white	1151	NS	In egg	0.00%		
		yolk	6252	NS	In egg	0.02%		

Country (Reported Year)	Investigated Year	Sample	No. of Samples	No. of eggs pooled	Object	% Positive	Serotype information (% of isolates)	Reference
Germany (2007)	2006	Eggs entire	4761	NS	Whole	0.59%	<i>S. Enteritidis</i> (81.4) <i>S. Typhimurium</i> (4.7) Other (4.7)	(Hartung, 2007b)
		Shells	3334	NS	On egg	0.39%		
		Egg white	575	NS	In egg	0.00%		
		yolk	3356	NS	In egg	0.06%		
Germany (2008)	2004-05	Eggs	80	10	On egg	1.25%	<i>S. Kimuenza</i> (100.0)	(Schwaiger <i>et al.</i> , 2008)
					In egg	0.00%		
Germany (2008)	2007	Eggs entire	6382	NS	Whole	0.72%	<i>S. Enteritidis</i> (58.5) <i>S. Typhimurium</i> (1.5) Other (36.9)	(Hartung, 2008)
		Shells	3212	NS	On egg	0.53%		
		Egg white	1347	NS	In egg	0.00%		
		yolk	3339	NS	In egg	0.06%		
Germany (2009)	2008	Eggs entire	7468	NS	Whole	0.25%	<i>S. Enteritidis</i> (87.5) Other (12.5)	(Hartung, 2009)
		Shells	6135	NS	On egg	0.21%		
		Egg white	305	NS	In egg	0.00%		
		yolk	5874	NS	In egg	0.02%		
Germany (2010)	2009	Eggs entire	5484	NS	Whole	0.33%	<i>S. Enteritidis</i> (80.3) Other (19.7)	(Hartung and Käsbohrer, 2010)
		Shells	2970	NS	On egg	0.30%		
		Egg white	125	NS	In egg	0.00%		
		yolk	2953	NS	In egg	0.03%		
India (2003)	NS	Eggs	534	1	On egg	9.55%	<i>Salmonella</i> spp. (100.0)	(Bajaj <i>et al.</i> , 2003)
					In egg	1.31%		
					Whole	10.86%		
India (2006)	1997-98	Unwashed eggs	492	1	On egg	6.10%	<i>S. Enteritidis</i> (89.7) <i>S. Cerro</i> (5.1) <i>S. Molade</i> (2.6) <i>S. Mbdanka</i> (2.6)	(Suresh <i>et al.</i> , 2006)
					In egg	1.83%		
India (2009)	NS	Eggs	110	1	NS	2.73%	<i>S. Heidelberg</i> (100)	(Lingaraja <i>et al.</i> , 2009)

Country (Reported Year)	Investigated Year	Sample	No. of Samples	No. of eggs pooled	Object	% Positive	Serotype information (% of isolates)	Reference
India (2010)	2006-07	Eggs	560	1	On egg	2.14%	<i>S. Typhimurium</i> (55.5) <i>S. Lagos</i> (22.2) Rough <i>Salm.</i> (14.8) <i>S. Africana</i> (3.7) <i>Salmonella</i> II (3.7)	(Singh <i>et al.</i> , 2010)
					In egg	2.14%		
					Whole	0.55%		
Iran (2010)	2008	Eggs	250	NS	On egg	1.6%	<i>S. Typhimurium</i> (100.0)	(Jamshidi <i>et al.</i> , 2010)
					In egg	0.00%		
Ireland (2007)	NS	Northern Ireland Eggs	2503	6	On egg	0.04%	<i>S. Infantis</i> (50.0) <i>S. Montevideo</i> (50.0)	(Murchie <i>et al.</i> , 2007)
					In egg	0.00%		
		Republic of Ireland Eggs	2515	6	On egg	0.00%		
					In egg	0.00%		
Japan (2002)	1998-99	Eggs	4398	5	In egg	0.00%		(Sunagawa <i>et al.</i> , 2002)
Japan (2008)	2004-06	Packed eggs	9010	19	In egg	0.03%	<i>S. Enteritidis</i> (81.2) <i>S. Infantis</i> (18.1) Other (0.7)	(Lapuz <i>et al.</i> , 2008)
		Processed eggs	11280	40	In egg	1.03%		
		Unprocessed eggs	1766	90	Whole	1.70%		
Japan (2010)	2007-08	Raw shell eggs	2030	10	On egg	0.25%	<i>S. Enteritidis</i> (40.0) <i>S. Derby</i> (20.0) <i>S. Livingstone</i> (20.0) <i>S. Cerro</i> (20.0)	(Sasaki <i>et al.</i> , 2010)
					In egg	0.00%		
Korea (2000)	1998	Shell eggs	135	1	On egg	0.00%		(Chang, 2000)
					In egg	0.00%		
Korea (2003)	1993-2001	eggs	40	NS	NS	0.00%		(Chung <i>et al.</i> , 2003)
Kuwait (2007)	2004-05	Hatching eggs	30	1	In egg	10.00%	<i>S. Enteritidis</i> (100.0)	(Al-Zenki <i>et al.</i> , 2007)
Poland (2001)	1997-98	Unwashed eggs	1200	1	On egg	0.00%		(Radkowski, 2001)
					In egg	0.00%		

Country (Reported Year)	Investigated Year	Sample	No. of Samples	No. of eggs pooled	Object	% Positive	Serotype information (% of isolates)	Reference	
Portugal (2004)	NS	Eggs	150	1	On egg	0.00%		(Verde <i>et al.</i> , 2004)	
					In egg	0.00%			
Thailand (2007)	2003	Chicken eggs	50	NS	NS	14.00%	<i>S. Braenderup</i> (22.2) <i>S. Cerro</i> (11.1) <i>S. Derby</i> (11.1) <i>S. Enterica</i> (11.1) <i>S. Hvitvingfloss</i> (11.1) <i>S. Idikan</i> (11.1) <i>S. Mbandaka</i> (11.1) <i>S. Montevideo</i> (11.1)	(Vindigni <i>et al.</i> , 2007)	
Trinidad and Tobago (2005)	NS	Table eggs	138	6	On egg	2.90%	<i>S. Enteritidis</i> (57.0) <i>S. Ohio</i> (18.9) <i>S. Mbdanka</i> (8.1) <i>S. Javiana</i> (6.7) <i>S. Braenderup</i> (4.1) <i>S. Caracas</i> (1.4) <i>S. Georgia</i> (1.4) Group C1 (1.4)	(Adesiyun <i>et al.</i> , 2005)	
					In egg	10.14%			
					Whole	13.04%			
Turkey (2002)	NS	Eggs	9	1	In egg	0.00%		(Mercanoglu and Aytac, 2002)	
UK (1998)	1996-97	Raw shell eggs	2090	6	On egg	0.38%	<i>S. Enteritidis</i> (33.3) <i>S. Infantis</i> (22.2) <i>S. Typhimurium</i> (11.1) <i>S. Kentucky</i> (11.1) <i>S. Mbandaka</i> (11.1) <i>S. Montevideo</i> (11.1)	(Wilson <i>et al.</i> , 1998)	
					In egg	0.05%			
UK (2005)	2003	Raw shell eggs	5686	6	Whole	0.30%	<i>S. Enteritidis</i> (88.2) <i>S. Typhimurium</i> (5.9) <i>S. Livingstone</i> (5.9)	(Elson <i>et al.</i> , 2005)	
		Origins of above samples							
		UK	4987		Whole	0.32%			
		Spain	22		Whole	0.00%			
		Germany	10		Whole	0.00%			
		Other countries	16		Whole	0.00%			
		Unknown	651		Whole	0.15%			

Country (Reported Year)	Investigated Year	Sample	No. of Samples	No. of eggs pooled	Object	% Positive	Serotype information (% of isolates)	Reference	
UK (2007)	2002	Raw shell eggs	726	6	Whole	0.96%	<i>S. Enteritidis</i> (85.7) Other (14.3)	(Little <i>et al.</i> , 2007a)	
		Origins of above samples							
		UK	541		Whole	0.18%			
		France	45		Whole	0.00%			
		Unknown	140		Whole	4.29 %			
UK (2007)	2005-06	Non-UK produced raw shell eggs	1744	6	On egg	9.00%	<i>S. Enteritidis</i> (84.9) <i>S. Mbandaka</i> (8.1) <i>S. Rissen</i> (1.2) <i>S. Braenderup</i> (0.6) <i>S. Infantis</i> (0.6) <i>S. Panama</i> (0.6) <i>S. Weltevreden</i> (0.6) Other (3.4)	(Little <i>et al.</i> , 2007b)	
		Origins of above samples				In egg			0.57%
		Spain	1157		Whole	13.31%			
		France	348		Whole	0.57%			
		Germany	45		Whole	0.00%			
		Other countries	194		Whole	0.52%			
UK (2008)	2005-06	Non-UK produced raw shell eggs	1588	6	On egg	0.38%	<i>S. Enteritidis</i> (83.3) <i>S. Mbandaka</i> (16.7)	(Little <i>et al.</i> , 2008)	
		Origins of above samples				In egg			0.06%
		UK	1413		Whole	0.35%			
		Spain	48		Whole	0.00%			
		Germany	38		Whole	2.63%			
		France	27		Whole	0.00%			
		Other countries	43		Whole	0.00%			
		Unknown	19		Whole	0.00%			
Uruguay (2010)	NS	Eggs	620	20	In egg	9.35%	<i>S. Enteritidis</i> (13.8) <i>S. Darby</i> (67.2) <i>S. Gallinarum</i> (15.5) <i>S. Panama</i> (3.4)	(Betancor <i>et al.</i> , 2010)	
US (2001)	NS	Shell eggs	5	NS	NS	20.00%	<i>S. Enteritidis</i> (100.0)	(Peng and Shelef, 2001)	
US (2004)	NS	Washed eggs	36	1	On egg	0.00%		(Musgrove <i>et al.</i> , 2004)	
		Unwashed eggs	36	1	On egg	0.00%			

Country (Reported Year)	Investigated Year	Sample	No. of Samples	No. of eggs pooled	Object	% Positive	Serotype information (% of isolates)	Reference
US (2005)	NS	Eggs before processing	60	5	On egg	25.00%	<i>Salmonella</i> spp. (100.0)	(Musgrove <i>et al.</i> , 2005)
		Eggs during processing	84	5	On egg	14.29%		
		Eggs after processing	54	5	On egg	12.96%		
US (2007)	NS	Restricted eggs	90	6	On egg	2.22%	<i>S. Heidelberg</i> (100.0)	(Jones and Musgrove, 2007)
					In egg	0.00%		
US (2009)	NS	Eggs	120	9	On egg	0.00%	<i>S. Infantis</i> (50.0) <i>S. Ohio</i> (50.0)	(Kretzschmar-McCluskey <i>et al.</i> , 2009)
					In egg	1.67%		

<sup>1</sup> *S. Enteritidis* only was analysed for

**Table 8: Prevalence of *Salmonella* in egg products**

Country (Reported Year)	Investigated Year	Sample	No. of Samples	No. of eggs pooled	Object	% Positive	Serotype information (% of isolates)	Reference
Canada (1998)	NS	Pooled egg products	21	NA	NA	0.00%		(Shaw <i>et al.</i> , 1998)
Canada (1998)	NS	Egg powders and liquid eggs	200	NA	NA	0.5%	<i>Salmonella</i> spp. (100.0)	(Blais <i>et al.</i> , 1998)
Japan (2006)	2003	Liquid whole eggs	14	NA	NA	0.00%	<i>S. Enteritidis</i> (100.0) <sup>1</sup>	(Kudo <i>et al.</i> , 2006)
		Liquid egg yolks	14	NA	NA	7.14%		
		Liquid egg whites	14	NA	NA	7.14%		
Japan (2007)	NS	Liquid eggs	627	NA	NA	0.96%	<i>S. Enteritidis</i> (100.0) <sup>1</sup>	(Lapuz <i>et al.</i> , 2007)
Japan (2009)	1992 - 2002	Commercial liquid eggs	1327	NA	NA	8.1%	<i>S. Enteritidis</i> (50) Wide variety of other serotypes	(Hara-Kudo and Takatori, 2009)
UK (2010)	NS	Pooled raw shell egg mix	7641	NS	NA	0.13%		(Gormley <i>et al.</i> , 2010a)
US (2010)	2009	Egg white	313	NA	NA	0.55%	<i>S. Enteritidis</i> (33.3) <i>S. Heidelberg</i> (33.3) Untypable (33.3)	(FSIS, 2010)
		Whole eggs or yolks (<2% added ingredients)	430	NA	NA	0.00%		
		Whole eggs with added yolk or whole egg blends	149	NA	NA	0.00%		
		Whole eggs or yolks (>2% added salt or sugar)	325	NA	NA	0.62%		
		Dried yellow egg products	111	NA	NA	0.00%		
		Spray dried egg whites	102	NA	NA	0.00%		
		Pan dried egg whites	11	NA	NA	0.00%		

<sup>1</sup> *S. Enteritidis* only was analysed for

**Table 9: Prevalence of *Salmonella* in layer hens**

Country (Reported Year)	Investigated Year	Samples	No. of Samples	% Positive	Serotype information (% of isolates)	Reference
EU (2006)	2004-05	Laying hen holdings	4797 (31 927 samples)	30.7%	<i>S. Enteritidis</i> (51.33) <i>S. Infantis</i> (8.31) <i>S. Typhimurium</i> (5.24) <i>S. Mbandaka</i> (4.44) <i>S. Livingstone</i> (2.75) <i>S. Virchow</i> (2.47) <i>S. Hadar</i> (2.00) <i>S. Ohio</i> (1.74) <i>S. Subspec. I Rauform</i> (1.61) <i>S. Braenderup</i> (1.52) <i>S. Montevideo</i> (1.38) <i>S. Agona</i> (1.18) <i>S. Tennessee</i> (1.12) <i>S. Bredeney</i> (0.98) <i>S. Anatum</i> (0.65) <i>S. Seftenberg</i> (0.60) <i>S. Newport</i> (0.54) <i>S. Kentucky</i> (0.48) <i>S. Indiana</i> (0.43) <i>S. Rissen</i> (0.43)	(EFSA, 2006)
		Origins of above samples by Member State				
		Austria	334	15.6%		
		Belgium	130	35.4%		
		Cyprus	2	50.0%		
		Czech Republic	64	65.6%		
		Denmark	85	2.4%		
		Estonia	11	18.2%		
		Finland	249	0.4%		
		France	511	17.2%		
		Germany	522	28.7%		
		Greece	107	37.4%		
		Hungary	267	43.8%		
		Ireland	146	1.4%		
		Italy	295	30.2%		
		Latvia	6	16.7%		
		Lithuania	8	50.0%		
		Luxembourg	9	0.00%		
		Poland	290	77.2%		
		Portugal	44	79.5%		
		Slovenia	19	19.4%		
		Spain	481	73.2%		
Sweden	97	0.00%				
The Netherlands	392	15.8%				
United Kingdom	413	11.9%				
Norway (voluntary)	236	0.00%				
<b>Country</b>	<b>Investigated</b>	<b>Samples</b>	<b>No. of Samples</b>	<b>% Positive</b>	<b>Serotype</b>	<b>Reference</b>

(Reported Year)	Year				information (% of isolates)	
Germany (2008)	2004-05	Cloacal swabs of layer hens	799	2.63%	<i>S. Kimuenza</i> (30.4) <i>S. Typhimurium</i> DT012 (21.7) <i>S. Typhimurium</i> RDNC (17.4) <i>S. Typhimurium</i> DT 104 L (4.3) <i>S. Enteritidis</i> (4.3) <i>S. Kentucky</i> (4.3) Serotype B (17.4)	(Schwaiger <i>et al.</i> , 2008)
		Organic laying farm	399	3.5%		
		Conventional laying farm	400	1.8%		
Turkey (2010)	NS	Layer hens	259	55.6%	<i>S. Enteritidis</i> (70.1) <sup>1</sup>	(Temelli <i>et al.</i> , 2010)
Portugal (2008)	NS	Layer Farms	30	23.0%	<i>Salmonella enterica</i> subsp. <i>enterica</i> (0:3.10) (67.0) <i>S. Mbdanka</i> (11.0) <i>S. Infantis</i> (11.0) <i>S. Newport</i> (11.0)	(Kottwitz <i>et al.</i> , 2008)
UK (2007)	2004-05	Environmental samples from layer farms	454	11.7%	<i>S. Enteritidis</i> (45.29) <i>S. Typhimurium</i> (12.9) <i>S. Mbandaka</i> (6.5) <i>S. Seftenberg</i> (4.8) <i>S. Agona</i> (3.2) <i>S. Corvallis</i> (3.2) <i>S. Cubana</i> (3.2) <i>S. Livingstone</i> (3.2) <i>S. Yoruba</i> (3.2) <i>S. Agoma</i> (1.6) <i>S. Havana</i> (1.6) <i>S. Infantis</i> (1.6) <i>S. Kentucky</i> (1.6) <i>S. Tennessee</i> (1.6) <i>S. Thompson</i> (1.6) <i>S. Virchow</i> (1.6) 4, 12:d:- (1.6) 61:-:1,5,7 (1.6)	(Snow <i>et al.</i> , 2007)
Country	Investigated	Samples	No. of Samples	% Positive	Serotype	Reference

(Reported Year)	Year				information (% of isolates)	
US (2000)	1999	Layer flocks	200 layer houses	7.1%	<i>S. Enteritidis</i> (100.0) <sup>1</sup>	(USDA, 2000)
US (2007)	NS	Layer hens	78	30.8%	<i>S. Kentucky</i> (62.0) <i>S. Montevideo</i> (11.0) Untypable (11.0) <i>S. Typhimurium</i> (var 5-)(4.0) <i>S. Heidelberg</i> (4.0) <i>S. Seftenberg</i> (2.0) Non-motile (2.0) 8,(20):-:z6 (2.0)	(Li <i>et al.</i> , 2007)

<sup>1</sup> *S. Enteritidis* only was analysed for

## 8 APPENDIX 2: EVALUATION OF ADVERSE HEALTH EFFECTS

Salmonellae possess virulence determinants enabling adhesion to small intestinal epithelial cells, provided they survive the low pH of the stomach and other innate immune host defence mechanisms (Jay *et al.*, 2003). After entering epithelial cells, pathogenic salmonellae may multiply within a protective vacuole. Disruption of cellular tight junctions, leading to paracellular passage of ions, water and immune cells together with induction of host inflammatory cells is likely to contribute to the production of diarrhoea (Haraga *et al.*, 2008).

Two serotypes that have caused major problems overseas are *S. Enteritidis*, which is capable of trans-ovarian transmission into eggs (especially phage type 4 (PT4)), and the antibiotic resistant *S. Typhimurium* definitive phage type 104 (DT104).

*S. Enteritidis* PT4 became the most prevalent *Salmonella* causing human infection in the UK during the 1980s and 1990s. This was, in part, due to the fact that chicken eggs can be infected with *S. Enteritidis* PT4 internally or externally by the time they are laid, or can subsequently become contaminated after lay (Advisory Committee on the Microbiological Safety of Food, 1993). Similar problems occurred in the US, but involved a wider range of phage types.

New Zealand does not appear to have a reservoir of the phage types associated with trans-ovarian egg contamination. The notified human cases of salmonellosis infected with *S. Enteritidis* PT4 have usually recently travelled overseas.

Antibiotic resistant *S. Typhimurium* DT104 is infrequently isolated from humans in New Zealand (52 isolates since 1992, including a small 3 case outbreak in 1997). Of the 52 human isolates 50 were multiresistant. Since 1992, this serotype has only been isolated on 7 occasions from non-human sources (1 environmental, 1 poultry feed, 1 poultry environment, 3 canine and 1 feline). Three of the non-human isolates have been multiresistant strains (Carolyn Nicol, ERL, personal communication, June 2011).

### 8.1 Dose-Response

Gastric hydrochloric acid is an important barrier to preventing *Salmonella* spp. ingested with food or water from surviving to invade the cells of the small intestine (Smith, 2003). However, the survival of salmonellae in the stomach is enhanced when they are ingested with fatty or proteinaceous foods, or the pH of the stomach acid is increased (e.g. by antacids, or by medical conditions or interventions) (Kothary and Babu, 2001; Smith, 2003). In a stimulated stomach, one strain of *S. Typhimurium* survived longer when digested with scrambled egg than with lettuce (Koseki *et al.*, 2010). The authors postulated that ingested bacteria in the stomach would barely be inactivated in the real digestive process. Furthermore, if previously exposed to acidic pH, salmonellae can develop acid resistance that helps them survive exposure to gastric acid (a response which also enhances their survival in low acid foods) (Smith, 2003).

#### 8.1.1 Dose-response from feeding trials

Results obtained through feeding studies usually indicate that consumption of a large number of organisms causes gastrointestinal disease. However, these studies are usually conducted by feeding the pathogens to healthy adult volunteers in liquids such as milk or sodium bicarbonate, and do not consider low doses (Bollaerts *et al.*, 2008; Kothary and Babu, 2001). Therefore,

the results do not necessarily indicate the true dose-response relationship for the variety of individuals found in a normal population, or for foodborne transmission.

A set of volunteer feeding studies reported in 1951 (McCullough and Eisele, 1951a;b;c;d), while criticised for various deficiencies (Oscar, 2004; WHO/FAO, 2002), are commonly used to predict dose-response for salmonellosis. These studies observed illness after healthy young men ingested different doses of six *Salmonella* serotypes. In a review of these studies, Kothary and Babu (2001) reported that the infective dose ranged between  $10^5$  and  $10^{10}$  organisms depending on the *Salmonella* serotype. The attack rate was also serotype-dependent, and ranged from 16-50%.

A number of studies have used the McCullough and Eisele data to model dose-response. One model, combining data only from cases who had not previously participated in the experiments, predicted that a dose of  $2.4 \times 10^4$  salmonellae would infect 50% of the population (FAO/WHO, 2002). Another approach investigated variability between the serotypes (Oscar, 2004). The minimum illness doses ranged from  $6.0 \times 10^4$  of a *S. Bareilly* strain to  $2.1 \times 10^9$  of a *S. Pullorum* strain. The minimum illness doses of the *S. Derby* and *S. Newport* strains were  $7.6 \times 10^6$  and  $1.7 \times 10^7$  organisms, respectively.

#### 8.1.2 Dose-response from outbreak data

In contrast to human feeding trials, data from outbreaks suggest that the infective dose could be as high as  $10^7$ - $10^9$  salmonellae, or less than 100 salmonellae (Kothary and Babu, 2001). A high fat or protein content in the food vehicle (e.g. ice cream, chocolate, cheese, beef, or egg) can help protect salmonellae from gastric acidity, and salmonellosis outbreaks often involve young children and the elderly who are more susceptible to *Salmonella* infection (Kothary and Babu, 2001; Waterman and Small, 1998). In a salmonellosis outbreak in the US caused by contaminated ice cream, a dose sufficient to cause disease may have been as low as 6 cells in 65 g of ice cream (Hennessy *et al.*, 1996). Similarly, ingestion of as few as 10 *S. Typhimurium* cells may have been sufficient to cause symptomatic disease in a US outbreak caused by contaminated chocolate (Wilson and Baker, 2009a). Across 31 outbreaks in Japan, the calculated dose causing infection ranged from 11 to  $7.5 \times 10^9$  CFU/person (or from 31 to  $3.8 \times 10^6$  cfu/person for the 13 outbreaks where food samples were definitely frozen before testing) (Kasuga *et al.*, 2004). An analysis of nine outbreaks in Japan suggested an inverse relationship between the dose ingested and the incubation period before symptoms become evident (Abe *et al.*, 2004).

Using outbreak data, FAO/WHO produced a dose-response model as an output from the joint risk assessments of *Salmonella* in eggs and broiler chickens (FAO/WHO, 2002). The model was based on 20 outbreaks in North America and Japan (12 *S. Enteritidis*, 3 *S. Typhimurium*, and one each of *S. Heidelberg*, *S. Cubana*, *S. Infantis*, *S. Newport* and *S. Oranienburg*) with vehicles of transmission that included meat, eggs, dairy products, cake, vegetables and water. For the ingestion of  $10^{10}$  cells there was a probability of around 0.9 (90%) of illness, while the ingestion of  $10^1$  cells resulted in a probability of around 0.02 (2%). The model also predicts that a dose of  $10^4$  cells has a probability of illness of 50%. Thus the probability of illness from exposure to small doses is low. For outbreaks where food contains only low numbers of organisms but has been widely consumed, a small proportion of consumers are likely to become ill but this may equate to a large number of cases.

The FAO/WHO model has been developed further to account for differences in host susceptibility, serotype infectivity and food matrix (Bollaerts *et al.*, 2008). The FAO/WHO model separated the cases in each outbreak into normal and susceptible populations (e.g. children) where possible, but concluded that there was insufficient evidence to show that some segments of the population have a higher probability of illness. However, Bollaerts *et al.* (2008) (using the same data set) concluded that a susceptible population has a higher probability of illness at low dose levels when the pathogen and food matrix combine to increase virulence (e.g. fatty foods). The authors' analyses also suggested that there is some immunity in the normal population but not in the susceptible population.

A recent study (Teunis *et al.*, 2010) used data from 35 salmonellosis outbreaks, three sporadic cases for which there was good dose information and two human volunteer feeding studies (the doses ranged from  $<10$  to  $10^{11}$  organisms). From this wider data set, the researchers predicted that the number of cells that need to be ingested to cause a 50% probability of illness was as low as 36.3, although the 95% percentiles were wide ( $0.69-1.26 \times 10^7$ ).

### 8.1.3 Serotypes causing disease in New Zealand

There were 11,554 New Zealand cases of salmonellosis reported for the period 2000 to 2009 for which the *Salmonella* serotype was available (Adlam *et al.*, 2010). *S. Typhimurium* was the reported cause of 58.2% of these cases and the next most frequently reported serotype was *S. Enteritidis* (8.8% of cases). When considering serotype and phage type, *S. Typhimurium* DT160 was most frequently reported (19% of cases).

Table 10 displays the peak years and total number of cases for serotypes that have caused 50 or more salmonellosis cases between 2000 and 2009. Together these 35 serotypes caused 80% (9,290) of the 11,554 cases.

The serotypes significantly associated with cases living in highly urban areas were *S. Infantis* ( $p < 0.001$ ) and *S. Typhimurium* DT160 ( $p < 0.05$ ). The serotypes significantly associated with cases living in highly rural areas were *S. Saintpaul* ( $p < 0.001$ ), *S. Brandenburg* ( $p < 0.01$ ) and *S. Typhimurium* DT101 ( $p < 0.05$ ) (Adlam *et al.*, 2010).

**Table 10: *Salmonella* serotypes that caused 50 or more cases over the years 2000 to 2009 – peak occurrence and total cases (Adlam *et al.*, 2010)**

<i>Salmonella</i> serotype	Peak occurrence <sup>1</sup>										Total cases <sup>2</sup>
	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	
Typhimurium DT160		+									2,147
Typhimurium DT1	+	+	+								729
Brandenburg	+	+									700
Typhimurium DT135	+	+									698
Typhimurium DT156	+	+									562
Infantis									+	+	523
Typhimurium DT101	+										505
Enteritidis PT9a	+	+									432
Typhimurium DT42	+										257
Saintpaul						+					249

Typhimurium DT12a								+				237
Typhimurium DT9	+											182
Typhimurium RDNC-May 06 <sup>3</sup>										+		154
Heidelberg		+										150
Virchow					+				+			141
Typhimurium DT74								+				139
Typhimurium DT23		+										138
Typhimurium RDNC					+						+	137
Mississippi								+				95
Enteritidis PT4	+		+									95
Thompson								+				92
Agona								+				92
Weltevreden		+						+				88
Montevideo			+	+								79
Mbandaka								+		+		76
Newport		+						+				68
Stanley										+		65
Enteritidis PT6a										+		62
Corvallis								+				61
<i>Salmonella</i> sp. 4,5,12:d :-								+		+		59
Typhimurium DT8										+		58
Enteritidis PT1		+										58
Enteritidis PT1b											+	57
Hadar	+	+	+									55
Typhimurium RDNC Aug-01					+							50

1. + denotes where number of cases exceeds ten year mean plus one standard deviation for a given serotype.
2. There were 232 cases caused by *S. Typhimurium* that did not have phage typing data available. These cases are excluded from this table.
3. *Typhimurium* RDNC is not a single serotype, but a grouping of serotypes. RDNC stands for 'reaction does not conform' and indicates that the isolate does not match any recognised serotypes. RDNC can sometimes be followed by the month and year of isolation.

#### 8.1.4 Antimicrobial resistance of New Zealand *Salmonella* strains

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.<sup>28</sup>

Resistance to each of the 12 antimicrobials tested and multiresistance to three or more of these is shown in Table 11 for human isolates, and Table 12 for non-human isolates (isolates from animal or environmental samples), for the years 2005 to 2009.

<sup>28</sup> 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 1 December 2010).

The percentage of human or non-human *Salmonella* isolates that demonstrate antimicrobial resistance is low each year (usually 5% or less). Between 2005 and 2009, the percentage of isolates from humans that was resistant to three or more antimicrobials was between 4.1 and 6.4 per year. For non-human isolates this range was 1.3-4.4%. When the human and non-human isolates are combined, the percentages that were fully susceptible to all 12 antimicrobials each year were high: 92.7% (2005), 93.4% (2006), 85.8% (2007), 90.1% (2008) and 92.1% (2009).

**Table 11: Antimicrobial resistance of a sample of New Zealand *Salmonella* isolates from humans, 2005-2009<sup>1</sup>**

Antimicrobial	Percent of isolates resistant each year (n=number tested)				
	2005 (n=318)	2006 (n=276)	2007 (n=267)	2008 (n=277)	2009 (n=235)
Ampicillin	4.1	4.4	6.7	5.1	5.5
Cephalothin	0.6	0.4	0.8	0.4	1.7
Chloramphenicol	1.9	2.9	1.5	0.7	3.0
Ciprofloxacin	0.3	0	0	0	0.9
Co-amoxiclav	0.3	0	0	0.4	1.7
Co-trimoxazole	1.9	2.2	1.5	1.4	2.1
Gentamicin	0.6	0	0	0.4	1.7
Nalidixic acid	5.7	4.7	5.2	6.1	3.8
Streptomycin	3.1	4.7	8.6	5.1	5.1
Sulphonamides	4.1	5.1	6.4	5.8	6.0
Tetracycline	5.0	5.8	9.0	6.9	4.7
Trimethoprim	1.9	2.2	1.5	1.8	2.1
Multiresistant to $\geq 3$ antimicrobials <sup>2</sup>	4.1	4.7	6.4	5.8	5.5

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 1 December 2010).
2. For all years, co-trimoxazole and trimethoprim resistance were counted as one resistance for the estimates of multiresistance. In 2009, ciprofloxacin and nalidixic acid resistance was counted as one resistance.

**Table 12: Antimicrobial resistance of a sample of New Zealand *Salmonella* isolates from animal and environmental samples, 2005-2009<sup>1</sup>**

Antimicrobial	Percent of isolates resistant each year (n=number tested)				
	2005 (n=298)	2006 (n=298)	2007 (n=206)	2008 (n=277)	2009 (n=180)
Ampicillin	0.3	0.3	1.9	0.7	0
Cephalothin	0	0	0.5	0.7	0
Chloramphenicol	0	0	0	0.4	0
Ciprofloxacin	0	0	0	0	0
Co-amoxiclav	0	0	0.5	0.7	0
Co-trimoxazole	0.3	0.3	1.0	0	0
Gentamicin	0.3	0	0	0	0
Nalidixic acid	0	0	0.5	0	0
Streptomycin	3.0	1.7	6.3	5.4	5.0

Sulphonamides	1.7	1.7	7.8	2.5	4.4
Tetracycline	2.7	2.4	3.9	1.8	3.3
Trimethoprim	0.3	0.3	1.0	0	0
Multiresistant to $\geq 3$ antimicrobials <sup>1</sup>	1.3	1.3	4.4	2.2	2.8

1. For all years, co-trimoxazole and trimethoprim resistance were counted as one resistance for the estimates of multiresistance. In 2009, ciprofloxacin and nalidixic acid resistance was counted as one resistance.

In each year from 2005 to 2009 *Salmonella* isolated from humans were significantly ( $p < 0.05$ ) more resistant to ampicillin and nalidixic acid than *Salmonella* isolated from other sources. *Salmonella* isolated from humans were also significantly ( $p < 0.05$ ) more resistant to chloramphenicol (2005, 2006 and 2009) and tetracycline (2006, 2007 and 2008) than *Salmonella* isolated from other sources. In 2005, 2006 and 2008 *Salmonella* isolates from humans were also significantly ( $p < 0.05$ ) more multiresistant than *Salmonella* isolated from other sources. However an analysis of trends in resistance published for the years 2004 to 2009 reported no significant ( $p < 0.05$ ) changes in resistance to any of the antimicrobials during that period (ESR, 2010).

For each of the years from 2005 to 2009, *Salmonella* isolates from salmonellosis cases reported to have travelled overseas were significantly more resistant to two or more antimicrobials than isolates from cases for whom no recent overseas travel was reported. Isolates from cases who had travelled overseas were commonly more resistant to nalidixic acid, sulphonamides, tetracycline and streptomycin.

One isolate of the internationally recognised multiresistant *S. Typhimurium* DT104 clone was isolated in each of 2005, 2007 and 2009, and three were isolated in 2006 (none were isolated in 2008). There was no information available on where the cases in 2005, 2006 and 2007 acquired their infections, and the case in 2009 did not have a history of overseas travel. In 2008 ESR began monitoring other internationally recognised multiresistant *S. Typhimurium* clones U302, DT12, DT120 and DT193. There was one isolate of U302 in 2008 (the case had travelled to Mexico and the isolate was multiresistant) and 11 isolates of U302 in 2009 (five of these were from non-human sources and no travel was reported by the six human cases). A fully susceptible DT12 isolate was identified in 2008 from a human case, and in 2009 isolates from two human samples were identified as DT12 and one human isolate as DT120; the cases recorded no travel history.

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. The first ciprofloxacin-resistant *Salmonella* identified in New Zealand was isolated in 2002 (ESR, 2006). From 2005 to 2009 only three isolates were identified as ciprofloxacin resistant, however between 3 and 6% of each year's isolates were nalidixic acid resistant and therefore could fail fluoroquinolone treatment if causing an extra-intestinal infection.

## 8.2 Case-control studies in New Zealand

Case-control studies of salmonellosis in New Zealand are summarised in Table 13. In addition to those case-control studies reported in Section 3.3.5, four other case-control studies of

salmonellosis in New Zealand have been conducted, but these did not specifically address eggs as a risk factor.

One suspected cause of the 2005 outbreak of *S. Saintpaul* infection was the washing of raw carrots with untreated stream water (Neuwelt *et al.*, 2006). Samples of the stream water contained a high coliform count (460 to 2400 per 100 ml) and *E. coli* (9.8 to 88 per 100 ml), but *Salmonella* was not isolated. Egg consumption was not one of the foods included in the case-control study.

The results of the case-control study for the 2005 outbreak of *S. Enteritidis* 9a infection associated consumption of food purchased for a premises serving Middle Eastern dishes with illness (Anonymous, 2005). However, no single food item was identified as being associated with infection; consumption of chicken, hummus, flat bread, lettuce, tomato, onions and cabbage were all significant. When logistic regression was used to control for confounding between food items, no food item was identified as a significant independent risk factor. *S. Enteritidis* 9a was not isolated from any of the food samples taken from the implicated premises, although *S. Orion* was isolated from tahini. However, consumption of chicken remained significantly associated with illness after excluding people who had consumed food from the implicated premises. Egg consumption was not specifically investigated.

The 2009 outbreak of *S. Typhimurium* DT1 infection was associated with consumption of watermelon purchased from a roadside stall from a grower in Gisborne (McCallum *et al.*, 2009). An environmental investigation revealed unhygienic conditions in the watermelon packhouse and a septic tank located near the watermelon growing area. *Salmonella* was not isolated from watermelon samples. Cases also had increased odds of exposure to ham, in particular ham purchased from a specific supermarket, but this association was not significant.

An outbreak of *S. Typhimurium* DT42 infection in 2008-09 was caused by contaminated flour (Lisa McCallum, ESR, personal communication). Twelve cases were hospitalised (no fatalities) and the majority of the cases resided in Canterbury (22/75) and Otago (17/75). An elevated significant OR was also found for a specific supermarket and brand of flour. Flour samples were collected and tested for *Salmonella* from open packets in the homes of cases (4/26 positive), unopened packets that had been on sale in retail outlets prior to withdrawal (2/41 positive) and retrieved/withdrawn flour (3/23 batches of flour positive). Contamination levels were estimated for 3 of the positive samples. *Salmonella* counts ranged from 1 per 300g to 1 per 50g.

**Table 13: Case-control studies of salmonellosis in New Zealand**

Year	<i>Salmonella</i> serotype	No. cases <sup>1</sup>	No. cases and controls <sup>2</sup>	Exposures associated with increased disease risk OR/mOR (95% confidence interval) <sup>3</sup>	Reference
2001	Typhimurium DT160	45	119 cases 235 controls	<ul style="list-style-type: none"> <li>• Direct handling of dead wild birds, mOR=12.28 (2.76-54.63)</li> <li>• Exposure to person with diarrhoea and vomiting (D&amp;V) in household in 3 days before illness, mOR=4.67 (1.21-18.05)</li> <li>• Exposure to person with D&amp;V in any setting in 3 days before illness, mOR=3.81 (1.53-9.49)</li> <li>• Exposure to person with D&amp;V in household in 28 days before illness, mOR=3.11 (1.13-8.54)</li> <li>• Exposure to person with D&amp;V in any setting in 28 days before illness, mOR=3.05 (1.64-5.69)</li> <li>• Consumption of food at a large gathering, mOR=2.44 (1.27-4.68)</li> <li>• Consumption of any fast food, mOR=1.69 (1.04-2.75)</li> </ul> After step-wise regression: <ul style="list-style-type: none"> <li>• Direct handling of dead wild birds, aOR=10.5 (2.3-47.5)</li> <li>• Exposure to person with D&amp;V 28 days before illness, aOR=2.8 (1.4-5.4)</li> <li>• Consumption of any fast food, aOR=1.7 (1.0-2.9)</li> </ul>	(Sneyd <i>et al.</i> , 2002; Thornley <i>et al.</i> , 2003)
2002-2003	Brandenburg	85	43 cases 43 controls	After multivariate analysis: <ul style="list-style-type: none"> <li>• Occupational contact with live or dead sheep or lambs during the 3 days prior to illness, OR=9.79 (1.69-190.38)</li> <li>• Having a household member who had occupational contact with sheep or lambs in the 3 days prior to illness or interview, OR=4.31 (1.26-21.33)</li> </ul>	(Baker <i>et al.</i> , 2007)
2005	Saintpaul	19	19 cases 57 controls	<ul style="list-style-type: none"> <li>• Eaten raw carrots during the 3 day period prior to illness or interview, OR=4.0 (1.35-12.01); mOR=7.3 (1.8-30.6)</li> </ul> After controlling for age and matching telephone number, aOR=2.86 (0.66-12.3), i.e. not significant.	(Neuwelt <i>et al.</i> , 2006)
2005	Enteritidis 9a	24	24 cases 72 controls	<ul style="list-style-type: none"> <li>• Eaten food from a Middle Eastern restaurant prior to illness, OR=10.2 (2.4-49.9)</li> </ul>	(Anonymous, 2005)
2008	Mbandaka	34	21 cases 63 controls	<ul style="list-style-type: none"> <li>• Chicken breast prepared at home from a specific processor, OR= 10.71 (1.50-118.52)</li> <li>• Eat chicken prepared away from home, OR=5.41 (1.67-18.38)</li> <li>• Eat eggs prepared away from home, OR=4.58 (1.10-19.07)</li> <li>• Eat eggs prepared away from home prepared using other method (not scrambled, omelette, fried, boiled, poached), OR=14.75 (1.28-728.09)</li> </ul> After multivariate logistic regression of all exposures where p-value was ≤0.05:	(McCallum and Das, 2008)

				<ul style="list-style-type: none"> <li>Chicken breast prepared at home, purchased from premise supplied by a specific processor, OR=9.24 (1.23-69.48)</li> <li>Eggs prepared away from home, OR=7.41 (1.67-32.99)</li> <li>Iceberg lettuce purchased from a specific supermarket, OR=6.25 (1.33-29.38)</li> </ul> <p>After multivariate logistic regression of all exposures where p-value was <math>\leq 0.05</math>, excluding 'chicken breast prepared at home, purchased from premise supplied by a specific processor':</p> <ul style="list-style-type: none"> <li>Chicken prepared away from home, OR=5.83 (1.83-18.52)</li> <li>Eggs prepared away from home, OR=6.11 (1.43-26.06)</li> </ul>	
2009	Typhimurium DT1	19	15 cases 40 controls	<ul style="list-style-type: none"> <li>Watermelon eaten at home, OR=5.17 (1.22-22.42)</li> <li>Watermelon purchased from roadside stall, OR=9.5 (1.08-114.8)</li> <li>Ate any water melon (at home or away from home), OR=6.00 (1.4-27.28)</li> </ul> <p>After controlling for age group and sex:</p> <ul style="list-style-type: none"> <li>Watermelon eaten at home, aOR=6.78 (1.26-36.59)</li> <li>Ate any water melon (at home or away from home), aOR=7.33 (1.36-39.61)</li> </ul> <p>After excluding cases that had contact with symptomatic people and controlling for age group and sex:</p> <ul style="list-style-type: none"> <li>Watermelon eaten at home, aOR=9.87 (1.46-66.79)</li> <li>Ate any water melon (at home or away from home), aOR=6.22 (1.12-34.68)</li> </ul>	(McCallum <i>et al.</i> , 2009)
2008-2009	Typhimurium DT42	75	33 cases 66 controls	<ul style="list-style-type: none"> <li>Eating, licking or tasting uncooked baking mixture, OR=3.6 (1.2-10.7)</li> </ul> <p>After adjusting for eggs in individual baking ingredients:</p> <ul style="list-style-type: none"> <li>Flour, aOR=5.7 (1.1-29.1)</li> </ul> <p>After adjusting for flour in individual baking ingredients:</p> <ul style="list-style-type: none"> <li>Eggs, OR=0.8 (0.2-3.4), i.e. not significant</li> </ul>	Lisa McCallum, ESR, personal communication

1. Number of cases initially identified in the outbreak or cluster.
2. Number of cases and controls included in the case-control study.
3. OR, odds ratio.  
mOR, matched odds ratio:  
aOR, adjusted odds ratio: Controlling for factors such as age, sex or other exposures.

### 8.3 Adverse health effects in other countries

The global burden of non-typhoid salmonellosis (circa 2006) was recently estimated at 93.8 million cases, with 155,000 deaths (Majowicz *et al.*, 2010). An estimated 80.3 million of these cases were from foodborne infection. The incidence was estimated as 1,140 per 100,000 person-years.

Table 14 shows the reported incidence of salmonellosis in several countries.

**Table 14: Reported incidence data for notified cases of salmonellosis in other countries\***

Country	Incidence (cases/100,000)	Year	Data source
Australia	43.6	2009	1
Canada	18.0	2006	2
EU total	23.7	2009	3
United Kingdom	23	2008	3
US	15.2	2009	4
US	16.9	2008	5
Fiji	5.1	2004-05	6

\* Does not include *S. Typhi* or *S. Paratyphi*

Data sources:

1. (National Notifiable Diseases Surveillance System, 2011)
2. (Public Health Agency of Canada, 2007)
3. (European Centre for Disease Prevention and Control, 2010)
4. (Matyas *et al.*, 2010). Data is based on ten US states.
5. (Hall-Baker *et al.*, 2010). Data is from health departments in the 50 states, five territories, New York City, and the District of Columbia.
6. (Dunn *et al.*, 2005)

The EU Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Foodborne Outbreaks in 2009<sup>29</sup> reported that the number of salmonellosis cases in humans decreased by 17.4 %, compared to 2008, and the statistically significant decreasing trend in the EU continued for the fifth consecutive year. In total 108,614 confirmed human cases were reported in 2009 and in particular, human cases caused by *S. Enteritidis* decreased markedly. The case fatality rate was 0.08 %. It is assumed that the observed reduction of salmonellosis cases is mainly attributed to successful implementation of national *Salmonella* control programmes in fowl populations; but also other control measures along the food chain may have contributed to the reduction.

Estimates of foodborne diseases acquired in the US have recently been reported for 31 major pathogens, including *Salmonella* spp. (Scallan *et al.*, 2011). The authors used data from a number of active and passive surveillance systems for the period 2000-2008, and based all estimates on the 2006 population. An estimated 9.4 million (90% credible interval 6.6-12.7 million) illnesses per year were domestically-acquired foodborne infections. Non-typhoidal *Salmonella* was the causative pathogen of an estimated 1 million (0.6-1.7 million), or 11%, of

<sup>29</sup> <http://www.efsa.europa.eu/en/efsajournal/pub/2090.htm> accessed 7 May 2011

these infections, second only to norovirus (5.5 million, 58%). Non-typhoidal salmonellosis was estimated to be the leading cause of hospitalisation due to domestically-acquired foodborne infection (35% of hospitalised cases) and deaths (28%).

Surveillance data from 1996-2000 have also been used to estimate the impact of foodborne disease in England and Wales (Adak *et al.*, 2005). Non-typhoidal salmonellosis was the estimated cause of 73,193/1,724,315 (4.2%) cases of domestically-acquired foodborne disease per annum, only exceeded by campylobacteriosis (19.6%), *Clostridium perfringens* infection (9.8%) or yersiniosis (7.5%). Salmonellosis was also estimated to be the leading cause of death (30%), and second only to campylobacteriosis in causing hospitalisation (12%). Poultry was also reported to be the vehicle for an estimated 0.5 million (29%) cases of domestically-acquired foodborne disease, and a case fatality rate of 38 per 100,000 cases.

In Australia, an estimated 81,000 (95% credibility interval (CrI) 23,000-138,000) cases of gastroenteritis per annum were caused by foodborne *Salmonella* infection (based on a typical year circa 2000; incidence 422.9 per 100,000 people<sup>30</sup>) (Hall *et al.*, 2005). These cases represented 5.5% of the total estimated cases of foodborne gastroenteritis caused by 16 known pathogens. An estimated 14,700 cases were hospitalised with foodborne gastroenteritis per year (including an estimated 11,000 cases infected with an unidentified pathogen), and of these, an estimated 1,060 (900-1,240; 7.2%) were caused by non-typhoidal *Salmonella* infection. In another study based on data from 2000-2004, the annual community incidence of salmonellosis in Australia was estimated as 49,843 (95% CrI 28,466-118,518) cases, and the salmonellosis rate as 262 (95% credible interval 150-624) per 100,000 people (Hall *et al.*, 2008).

### 8.3.1 *Salmonella* serotypes causing disease in other countries

The ten most frequently reported serotypes isolated from in 2008 have been reported for 26 EU member states (EFSA, 2010) (Table 15). *S. Enteritidis* and *S. Typhimurium* were the serovars most frequently associated with human illness. *S. Enteritidis* cases were most commonly associated with the consumption of contaminated eggs and poultry meat, while *S. Typhimurium* cases were mostly associated with the consumption of contaminated pig, poultry and bovine meat. The proportion of *S. Enteritidis* and *S. Typhimurium* cases with phage type data was very low (12.2% and 20.2%, respectively). The available data showed the most commonly identified *S. Enteritidis* phage types to be PT4 and PT8. *S. Typhimurium* U292 was the most commonly identified *S. Typhimurium* phage type (all were from Denmark). There are reports that salmonellosis caused by *S. Enteritidis* infection are declining as a result of vaccination programmes to immunise layers against *S. Enteritidis* (e.g. (Cogan and Humphrey, 2003; Collard *et al.*, 2008; Kornschober *et al.*, 2009)).

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<sup>30</sup> Calculated from a population of 19,153,400 as reported for June 2000, by the Australian Bureau of Statistics (<http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/3101.0Jun%202010?OpenDocument>, see Table 1; accessed 8 February 2011)

**Table 15: Ten most commonly confirmed human salmonellosis serotypes in the EU, 2008**

<i>Salmonella</i> serotype	N	%
Enteritidis	70,091	58.0
Typhimurium	26,423	21.9
Infantis	1,317	1.1
Virchow	860	0.7
Newport	787	0.7
Agona	636	0.5
Derby	624	0.5
Stanley	529	0.4
Bovismorbificans	501	0.4
Kentucky	497	0.4
Other	18,495	15.3
<b>Total</b>	<b>120,760</b>	-

Source: (EFSA, 2010); data submitted from 26 EU member states.

In Australia during 2009, the most commonly notified *Salmonella* serotype was *S. Typhimurium*, which was responsible for approximately 41% of all notified infections (serotype information was available for 6,983/7,464 (94%) *Salmonella* notifications) (OzFoodNet Working Group, 2010). Commonly isolated phage types during this year were *S. Typhimurium* DT170/DT108 and *S. Typhimurium* DT135/DT135a. *S. Enteritidis* is not endemic in Australian egg layer flocks and during 2009, 508 of the 587 (87%) cases of *S. Enteritidis* infection had reported overseas travel (no travel histories were available for 40 of the 587 cases).

Preliminary data for 2009 released by the Foodborne Diseases Active Surveillance Network (FoodNet) (based on data from ten US states) showed that among 6,371 *Salmonella* isolates serotyped, 10 serotypes accounted for 73.1% of infections (Matyas *et al.*, 2010). *S. Enteritidis* was most often identified (1,226, 19.2%, followed by *S. Typhimurium* (1,024, 16.1%), *S. Newport* (772, 12.1%) and *S. Javiana* (544, 8.5%).

The most frequently isolated serotypes in Canada during 2006 were *S. Enteritidis* (1,344/5,870 notifications where serotyping information was available, or 23%), *S. Typhimurium* (1,005, 17%) and *S. Heidelberg* (707, 12%) (Public Health Agency of Canada, 2007).

### 8.3.2 Salmonellosis outbreaks in other countries

Salmonellosis is a significant contributor to infectious intestinal disease outbreaks in many countries as shown by the data summarised in Table 16.

**Table 16: Foodborne outbreaks in other countries: Proportion attributed to *Salmonella* infection**

Country	Year(s)	Foodborne outbreaks attributed to <i>Salmonella</i> infection	Data source
Australia	2009	59/163 (36%)	(The OzFoodNet Working Group, 2010)
England and Wales	1992-2008	1,135/2,429 (47%), 114/928 (12.3%) attributed to eggs	(Gormley <i>et al.</i> , 2010b)
European Union	2008	490/890 (55%) Verified 1,888/5,332 (35%) All	(EFSA, 2010)
Japan	1981-95	17.2% of cases of known cause, 23.8% of outbreak cases (16.2% were of unknown cause)	(Lee <i>et al.</i> , 2001)
Korea	1981-95	28.3% of outbreaks of known cause, 31.2% of outbreak cases (26.6% were of unknown cause)	(Lee <i>et al.</i> , 2001)
Netherlands	1991-94	15.5% of outbreaks of known cause (90.4% were of unknown cause)	(Simone <i>et al.</i> , 1997)
Sweden	1992-97	17.8% of outbreaks of known cause, 14.5% of outbreak cases (61% of outbreaks were of unknown cause)	(Lindqvist <i>et al.</i> , 2000)
US	2007	142/1097 (12.9%)	(Boore <i>et al.</i> , 2010)

### 8.3.3 Outbreaks in Australia associated with eggs

Despite surveys indicating a low or nil prevalence of contamination on or in eggs in Australia, a number of outbreaks of salmonellosis in that country have been linked with contaminated eggs.

A marked increase in cases of salmonellosis due to *S. Typhimurium* 135 prompted an outbreak investigation in early 1991 in Victoria, Australia (Carnie *et al.*, 1991). Initial interviews suggested a common element was the consumption of Italian ice creams (gelati). Subsequent investigations included sampling of ice creams, eggs, and other foods at an implicated café. The eggs used to make the gelati appeared to be the most likely source of contamination as they were cracked, and the problem was compounded by freezer breakdowns allowing the ice cream to thaw.

Investigation of an outbreak of *S. Typhimurium* PT135a following a Christmas function in South Australia in 2001 found that consumption of sausage rolls, tirimasu, and chocolate slice were associated with increased risk (Hall, 2002). Low numbers of the bacteria were cultured from a number of leftover foods, but >1000 cfu/g were found in the tirimasu, which included raw eggs. It was thought that cross contamination from the tirimasu to other foods had occurred to provide the association with infection. Another outbreak, of the same serotype, in Perth in 2000 was circumstantially linked to a mock ice cream dessert containing raw eggs (Sarna *et al.*, 2002). The eggs had come from a farm where visually dirty eggs were soaked in a water tank for an unspecified time to clean them. A further outbreak of *S. Typhimurium* PT135

infection linked to the consumption of foods containing raw shell eggs in an aged care facility occurred in 2001 (Tribe *et al.*, 2002). The foods involved were a potato topping on a meat pie which was only lightly browned, and eggs whisked into a rice pudding immediately prior to serving. The eggs were traced to a single farm, where environmental sampling failed to reveal a specific source, but the serotype was identified in chicken manure.

More recently, an outbreak of *S. Typhimurium* PT44 infection in an aged care facility was linked to eggs sourced from a local farm (Roberts-Witteveen *et al.*, 2009). The eggs were supplied by a local free range farm and were consumed raw in a chocolate mousse. The eggs were “seconds”, with some being cracked and dirty, and the same strain of *Salmonella* was isolated from the shell of an unused egg.

An outbreak of infection with *S. Typhimurium* PT44 in a restaurant in Canberra in December 2008 was investigated by a case-control study (Dyda *et al.*, 2009). A strong association between illness and consumption of eggs and hollandaise sauce was found, and this was supported by environmental results. This included higher than recommended temperatures in the main storage fridge, and heating the hollandaise sauce under a heat lamp for softening before being used for multiple meals, both of which may have permitted bacterial growth. Further, it was found that the eggs themselves were not suitable for packing as first grade eggs and were intended to be processed into liquid pulp eggs. A packing error resulted in the eggs being boxed and sold. A further outbreak in a Canberra restaurant in February 2009, involved infection with *S. Typhimurium* PT170 (Reynolds *et al.*, 2010). Eating a tirimasu dessert containing raw eggs was a significant risk factor, but microbiological evidence could not be obtained from the restaurant, or from traceback investigations.

A series of outbreaks of salmonellosis due to *S. Typhimurium* PT197 in Queensland from 2006-2007 were traced by genotyping to a single egg producer (Slinko *et al.*, 2009). The producer was found to be selling cracked and dirty eggs. The producer’s eggs were withdrawn from sale, and no further outbreak strain cases were notified.

An examination of outbreaks occurring in long term care facilities in Australia found that in 4/9 outbreaks of salmonellosis in which a vehicle was identified, raw eggs were the source (Kirk *et al.*, 2011).

Common elements in these outbreaks are the consumption of raw or undercooked eggs, and the use of dirty and/or cracked eggs.

A study in Adelaide examined *Salmonella* serotypes found in human cases, retail poultry, and eggs, as well as the descriptive epidemiology of the cases between February and July 2008 (Fearnley *et al.*, 2011). Of the 94 participants in the study, 47.9% were notified with a *Salmonella* serotype that was also identified in the retail chicken and egg survey, and 62.2% of participants ate either chicken or eggs or both in the week before illness onset. However, due to the study design, a direct causal link could not be established. No *Salmonella* were found in the contents of the eggs, but 3.5% of the eggs were contaminated with *Salmonella* on the shell. In contrast, the prevalence of *Salmonella* on retail chicken was 38.8% (*S. Sofia* was 13.8% of total isolates and was not isolated from human cases).

### 8.3.4 Other overseas outbreaks and case-control studies

A national case-control study in France identified consumption of raw or undercooked egg containing foods as a statistically significant risk factor for *S. Enteritidis* infection in children aged 1-5 years (Delarocque-Astagneau *et al.*, 1998). Foods included mayonnaise and chocolate mousse. Eating soft boiled eggs, or hard boiled eggs cooked for less than 8 minutes were also risk factors.

A case-control study in 1989–1990 to examine exposures for *S. Enteritidis* and *S. Typhimurium* infections in Minnesota adults found that sporadic cases were significantly more likely to have consumed undercooked eggs, or eggs-containing foods during the three days before onset of illness (Hedberg *et al.*, 1993). While this result was not a surprise, it demonstrated an elevated risk from eggs despite no egg-associated outbreaks of salmonellosis being recognised in the region.

A case-control study of sporadic *Salmonella* food poisoning in the South East Wales area from July 1997 – December 1998 focused on domestic kitchen food handling risk factors (Parry *et al.*, 2002). In multivariate analysis it was found that any consumption of raw eggs and handling of free range eggs were significant risk factors.

A case-case analysis of *S. Enteritidis* sporadic cases in the US, compared to cases infected with other serotypes showed that *S. Enteritidis* infection was linked with international travel, consumption of chicken outside the home, and consumption of undercooked eggs prepared outside the home (Voetsch *et al.*, 2009).

A review of 497 *S. Enteritidis* PT4 outbreaks in England and Wales from 1992 – 2002 showed that eggs, egg products, and raw shell eggs were strongly associated with the pathogen, and cross contamination and inadequate heat treatment were the risk factors for most outbreaks (Gillespie *et al.*, 2005).

## 8.4 Risk assessments overseas

The FAO and WHO (2002) have jointly carried out a quantitative risk assessment (QRA) of *Salmonella* spp. in eggs and broiler chickens. The assessment was published in 2002<sup>31</sup>.

The risk assessment had several objectives;

- “1. To develop a resource document of all currently available information relevant to risk assessment of *Salmonella* in eggs and broiler chickens and also to identify the current gaps in the data that need to be filled in order to more completely address this issue.
2. To develop an example risk assessment framework and model for worldwide application.
3. To use this risk assessment work to consider the efficacy of some risk management interventions for addressing the problems associated with *Salmonella* in eggs and broiler chickens.”

The emphasis of the egg component of this risk assessment is on *S. Enteritidis*, and therefore it is not directly applicable to New Zealand.

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<sup>31</sup> <http://www.who.int/foodsafety/publications/micro/salmonella/en/index.html> accessed 16 May 2011

The risk assessment published by the US FSIS and the FDA in 1998 was updated and republished in 2006 (Schroeder *et al.*, 2006). Based on newly available data and improved modelling techniques, the model estimated that if all shell eggs produced in the US were pasteurised for a 3- $\log_{10}$  reduction of *S. Enteritidis*, the annual number of illnesses from *S. Enteritidis* in eggs would decrease from approximately 130,000 to 40,000. Pasteurization for a 5- $\log_{10}$  reduction of *S. Enteritidis* was estimated to reduce the annual number of illnesses to 19,000. The model also estimated that if all eggs produced in the US were stored and held at 7.2°C within 12 hours of lay, the annual number of illnesses from *S. Enteritidis* in eggs would decrease from 130,000 to 28,000. As a result, rapid cooling and pasteurization of shell eggs were predicted to be highly effective mitigations for reducing illnesses from consumption of *S. Enteritidis* in shell eggs. A more detailed evaluation of the effectiveness of pasteurisation in reducing illnesses using this model has also been published (Latimer *et al.*, 2008).

The European Food Safety Authority (EFSA) also reported a quantitative risk assessment of *S. Enteritidis* in shell eggs in Europe (EFSA, 2010). Prevalence values in terms of the number of eggs contaminated (internally and externally) with *S. Enteritidis* per million were presented based on a 2 stage Bayesian model. The first stage estimated the average flock prevalence over laying period in the production system and the second stage estimated the proportion of contaminated eggs for an infected flock. These stages were combined to give the expected number of infected eggs per million using the hen data from two European member states. The report found that the relationship between hen prevalence and egg prevalence in an infected flock could not be estimated accurately so that even if hen prevalence would be known exactly (which is not the case), the egg prevalence would still be quite uncertain to predict from the hen prevalence even though a correlation exists. It was recommended that interpretation and extrapolation of the results from the risk assessment must be done with caution as the results are sensitive to the data used and may be biased. Further revision of this risk assessment would require more data from other member states as well as data from hens and eggs from the same flock (with flock sizes) for some member states.

Prior to the finalisation of the FSANZ Standard for Eggs and Egg Products, a risk assessment was conducted.<sup>32</sup> This addressed both chemical and microbiological hazards, but concluded that *Salmonella* is the principal microorganism of human health concern associated with eggs and egg products. The quantitative risk assessment for *Salmonella* in eggs by FSANZ was largely drawn from a quantitative model for non *S. Enteritidis* serotypes in eggs developed for the Australian Egg Corporation Ltd (Thomas *et al.*, 2006). A copy of this model was not able to be obtained by the authors of this Risk Profile.

## 9 APPENDIX 3: CONTROL MEASURES IN OTHER COUNTRIES

### 9.1 United States

In July 2010, the final rule regarding egg safety developed by the FDA went into effect<sup>33</sup>. Under the rule, egg producers whose shell eggs are not processed with a treatment, such as pasteurisation, must:

- Buy chicks and young hens only from suppliers who monitor for *Salmonella* bacteria
- Establish rodent, pest control, and biosecurity measures to prevent spread of bacteria throughout the farm by people and equipment. Conduct testing in the poultry house for *Salmonella* Enteritidis. If the tests find the bacterium, a representative sample of the eggs must be tested over an eight-week time period (four tests at two-week intervals); if any of the four egg tests is positive, the producer must further process the eggs to destroy the bacteria, or divert the eggs to a non-food use
- Clean and disinfect poultry houses that have tested positive for *Salmonella* Enteritidis
- Refrigerate eggs at 45 degrees F during storage and transportation no later than 36 hours after the eggs are laid (this requirement also applies to egg producers whose eggs receive a treatment, such as pasteurisation).
- To ensure compliance, egg producers must maintain a written *Salmonella* Enteritidis (SE) prevention plan and records documenting their compliance. Egg producers covered by this rule must also register with the FDA.

Producers who sell all their eggs directly to consumers or have less than 3,000 hens are not covered by the rule.

The prevention plan is required to include:

- Documentation that pullets were raised under “SE-monitored” conditions;
- Records documenting compliance with the SE prevention measures, as follows:
  - Biosecurity measures.
  - Rodent and other pest control measures.
  - Cleaning and disinfection procedures performed at depopulation.
  - Refrigeration requirements.
  - Environmental and egg sampling procedures;
  - Results of SE testing;
    - Diversion of eggs;
    - Eggs at a particular farm being given a treatment; and
    - Records of review and of modifications of the SE prevention plan and corrective actions taken.

Given that this rule specifies *S. Enteritidis*, it is primarily focused on preventing trans-ovarian transmission, and growth within the egg during storage. The epidemic of *S. Enteritidis* infections from eggs in the US during the 1990s was brought under control by three factors: (1) farm based control measures to control the introduction of the bacterium into flocks (2) early and sustained refrigeration of shell eggs and (3) education of consumers and food workers regarding the risk of consuming raw or undercooked eggs (Braden, 2006). In this article, the

<sup>33</sup> <http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/EggSafety/EggSafetyActionPlan/ucm170615> accessed 26 July 2010

low prevalence of egg contamination (perhaps 1 in 20,000) was noted as being misleading since there are so many eggs produced (approximately 65 billion per year).

## 9.2 United Kingdom

Since 2004, UK regulations have required all Class A eggs (i.e. those sold through retail and to catering) to be marked with a code identifying the method of production, country of origin and the production establishment.

A *Salmonella* control programme in layer flocks was implemented in January 2008 in the UK<sup>34</sup>, as part of a larger programme to control *Salmonella* also in broilers and pigs<sup>35</sup>. The aim of the programme is to reduce the prevalence of *Salmonella* in flocks on holdings in the UK producing eggs for human consumption at least to the target levels set out in EU regulations. This would represent an annual reduction of at least 10% in the number of positive adult laying flocks compared with the previous year.

All layer flocks of 350 birds or more are included. Operators are required to implement a sampling programme. Samples for the detection of *Salmonella* are taken from day-old chicks to be reared for the production of eggs for human consumption, approximately 2 weeks before the birds come into lay, or before being moved to laying accommodation, and then at 15 weeks intervals during the egg laying phase, with the first sample taken when the birds are 22 to 26 weeks of age.

This control programme is in addition to an existing programme monitoring breeding flocks which was introduced in 2007<sup>36</sup>. This covers breeding flocks of 250 birds or more. Samples for the detection of *Salmonella* are taken from day-old chicks to be used for breeding, when the birds are approximately 4 weeks of age, and approximately 2 weeks before the birds come into lay.

A UK industry initiative, the British Lion mark, is used to denote eggs produced to a Code of Practice<sup>37</sup>. The Lion Quality Code of Practice was launched in 1998 and includes compulsory vaccination against *Salmonella* Enteritidis of all pullets destined for Lion egg-producing laying flocks, independent auditing, full traceability of hens, eggs and feed and a "best-before" date stamped on the shell and pack, as well as on-farm stamping of eggs and packing station hygiene controls. British Lion eggs account for more than 85% of UK egg production.

## 9.3 Australia

The Primary Production and Processing Standard for Eggs and Egg Products was gazetted by Food Standards Australia New Zealand (FSANZ) on 26 May 2011<sup>38</sup>. This Standard (which applies in Australia but not New Zealand) is intended to reduce the incidence of foodborne illness from *Salmonella* (the main microbiological hazard for eggs) by minimising the prevalence and concentration in eggs and egg products. In addition, it addressed the problem of egg traceability, which hinders the investigation of egg related illness.

<sup>34</sup> <http://www.salmonella.org.uk/layer/ncp.aspx> accessed 21 June 2011.

<sup>35</sup> <http://archive.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/zoonoses/ncp.htm> accessed 21 June 2011.

<sup>36</sup> <http://archive.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/zoonoses/documents/salmonella-breeders.pdf> accessed 21 June 2011

<sup>37</sup> <http://www.lioneggs.co.uk/> accessed 21 June 2011.

<sup>38</sup> <http://www.foodstandards.gov.au/foodstandards/proposals/proposalp301primaryp3426.cfm> accessed 30 June 2011

At the primary production stage, egg producers are required to identify and control the food safety hazards associated with the production of eggs. Specific requirements have been included for:

- the control of inputs
- waste disposal
- health and hygiene
- ensuring producers have the necessary food safety skills and knowledge
- the design, construction and maintenance of premises, equipment and transportation vehicles
- bird health
- traceability of eggs including a requirement for individual eggs to be marked with the producers' unique identification
- sale or supply of unsuitable eggs and egg pulp.

At the processing stage, egg processors are required to identify and control the food safety hazards associated with the processing of eggs and egg products. Specific requirements have been included for:

- receiving unacceptable eggs
- control of inputs
- waste disposal
- ensuring persons engaged in egg processing have the necessary food safety skills and knowledge
- design, construction and maintenance of premises, equipment and transportation vehicles
- traceability of eggs and egg products
- processing of egg products
- storage and transport of processed egg product
- sale or supply of unacceptable eggs or egg product.

The requirement that individual eggs are stamped on their shell raised concerns for several submitters on the proposed standard, given the increased costs, particularly for small producers.

In relation to storage, the Final Assessment Report for the Standard stated: “The Risk Assessment considered the additional information provided at Draft Assessment on outbreaks of egg related illness and concluded that there remains very little epidemiological data to implicate clean, intact eggs as the source of egg-associated illness and the prevalence of *Salmonella* contaminated eggs in Australia is very low (imported raw shell eggs for food are not permitted). Therefore, temperature, and time at that temperature, of shell eggs is important to ensure quality but is not a key factor in ensuring safety. Additionally, there are limitations on shell egg shelf life for quality reasons and current industry practice is to recommend that eggs are stored chilled.”

The Risk Assessment quotes the current Australian Egg Industry Code of Practice (CoP) (2005) as recommending a best-before date for packaged eggs of up to five weeks from the time of lay and storage at retail at <20°C. If stored at retail at >20°C the CoP recommends the eggs should be stored no longer than 4 days prior to sale.

#### 9.4 Spain

The use of Spanish eggs was identified as a significant risk factor in many *S. Enteritidis* (non PT4) outbreaks in England and Wales during 2002-2004 (Martelli and Davies, 2011). A study of *Salmonella* in Spanish feed mills has found that cotton seeds had the highest risk of contamination, and non-pelleted feed was eight times more likely to be contaminated than pelleted feed, due to the heat treatment involved in the production of pellets (Torres *et al.*, 2011).