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Horizontal Transfer and Growth of *Salmonella enterica* in Chicken (*Gallus gallus*) Eggs in New Zealand

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Requests for further copies should be directed to:

Specialist Adviser Food Processing
Ministry for Primary Industries
PO Box 2526
WELLINGTON 6140

Email: animalproducts@mpi.govt.nz
Telephone: 0800 00 83 33
Facsimile: 04-894 0300

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Horizontal Transfer and Growth of *Salmonella enterica* in Chicken (*Gallus gallus*) Eggs in New Zealand

Scientific Interpretive Summary

The Ministry for Primary Industries (MPI) risk management programme template (2004) was designed to assist all New Zealand egg producers to meet the requirements of the Animal Products Act 1999. The template specifies industry-agreed storage and shelf-life options based upon the Egg Producers Federation of New Zealand Inc (EPF) Code of Practice (2002) and on the findings of a range of scientific studies. The options for storage time for eggs from date of lay as specified in the risk management template are:

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

The premis on which the requirements were developed was that over time and given favourable conditions, any *Salmonella* present on the shell of the egg can penetrate the egg and grow to unacceptable levels, and that time/temperature factors can be manipulated to reduce the risk to the consumer.

Recently, the EPF has raised concerns that the science behind the requirements, and hence the requirements themselves, may be outdated. Specifically, it has been suggested that?:

- Australia allows 42 day date marking if eggs are stored or held at 15˚C or less;
- Audits of the retail sector in New Zealand show poor compliance with the Ne Zealand storage requirements;
- There is minimal evidence of foodborne illness attributable to eggs in New Zealand;
- *Salmonella* contamination of egg yolks does not occur and the level of contamination on the shells of eggs in New Zealand is low;
- Data from overseas studies based on *Salmonella* Enteritidis (SE) may not be relevant to non-SE strains that predominate in New Zealand.

This project was undertaken to examine these assertions with respect to horizontal transfer and growth of *Salmonella* in chicken eggs, and to ensure that risk management decisions can be made with reference to relevant and current scientific knowledge.

There is insufficient data to enable a quantitative risk assessment to be carried out to adequately compare risk estimates for alternative extended storage regimes. Nevertheless a qualitative assessment has been carried out with available data.

While there is no evidence that eggs are causing a significant contribution to human foodborne illness in New Zealand, the comparatively high level of contamination on the outside of eggs compared with Australia at the time of the most recent survey (2007), indicates that it is important to maintain food safety controls during processing and subsequent storage and handling. This is particularly so given the recent increase in egg related food safety incidents in Australia where contamination is supposedly lower, but date mark periods are extended by an additional seven days.

The egg itself possesses several defence mechanisms that reduce the likelihood of penetration of *Salmonella* from the outside into the egg. However, studies defining their effectiveness and, more importantly, which factors need to be controlled to prevent penetration during

processing and storage/handling are equivocal. Assurances of safety of eggs cannot, therefore, rely on these defence mechanisms and known processing factors.

Should the defences be breached, yolk mean time (YMT) defines how long eggs can be stored at a given temperature before the conditions inside 20% of those eggs would permit the growth of *Salmonella* to levels known to cause illness in humans. While validation of the YMT model for non-*S.* Enteritidis serovars is limited, evidence that initiation of growth of some non- *S.* Enteritidis serovars may occur at a much quicker rate than the YMT model predicts, thereby potentially underestimating the risk to human health of eggs contaminated with these serotypes. Nevertheless, the model demonstrates that a rapid reduction in YMT and increase in growth rate as the storage temperature rises.

Notwithstanding the data gaps and uncertainty in the evidence currently available, there is sufficient scientific evidence to suggest that contamination levels in eggs will increase if eggs are stored at >15°C for more than 21 days, or if the eggs are held at, or closely below, 15°C for more than 35 days. It would therefore appear prudent to maintain the current requirements for handling and storage of eggs.

It is also important to recognize that while temperature is an important determinant for the storage life of eggs, other factors such as the use of vaccination of flocks and implementation of HACCP-based risk management programmes (RMP) along the food chain will affect the ultimate risk to the consumer.

The uncertainties identified in this report necessitate the following further research:

- Evaluation of the outcomes of current international projects on the penetration of egg defences.
- Understanding the effects on *Salmonella* carriage of contemporary egg-handling procedures and pathogen control measures used in New Zealand egg production, e.g. use of vaccination, use of supplier guarantees for feeds, and the effect of ever-increasing free-range farming practices
- Consideration of a contemporary comprehensive baseline survey of *Salmonella* on the surface of eggs.

MPI will consider amending this assessment and recommend additional options if scientific evidence supporting an alternative approach becomes available.

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Executive Summary

Poultry and poultry products can carry *Salmonella* and have been associated with outbreaks of salmonellosis throughout the world.

While there is no evidence that eggs provide a significant contribution to human foodborne illness in New Zealand, the comparatively high level of contamination on the outside of eggs compared with Australia at the time of the most recent surveys (2007), indicates that it is important to maintain food safety controls during processing and subsequent storage and handling. This is particularly so given an apparent increase in egg related food safety incidents in Australia where contamination is supposedly lower, but date mark periods are extended.

This review was undertaken to examine the ability of *Salmonella* on egg shells to penetrate the shell and grow during storage, and to determine whether current New Zealand handling and storage requirements are justifiable.

The egg possesses several defence mechanisms that reduce the likelihood of penetration of *Salmonella* from the outside into the egg. However, studies defining their effectiveness and, more importantly, which factors need to be controlled to prevent penetration during processing and storage/handling are equivocal. Assurances of safety of eggs cannot, therefore, rely on these defence mechanisms and processing factors.

Should the defences be breached, yolk mean time (YMT) defines how long eggs can be stored at a given temperature before the conditions inside 20% of those eggs would permit the growth of *Salmonella*. There is evidence that growth of some non- *S*. Enteritidis serovars may be supported much more quickly than the YMT model predicts, indicating that this model may not be equally applicable to all serovars. Validation of the YMT model has been very limited in the case of non-*S*. Enteritidis serovars, which are known to be present in New Zealand layer environments.

It is possible therefore that use of the YMT may produce 'fail dangerous' predictions for strains of *Salmonella* contaminating eggs in New Zealand. For this reason, use of YMT cannot be considered an overly conservative approach in New Zealand, and the initiation of growth may occur more rapidly than predicted by the model. However, despite a need for better data to fill identified gaps, the YMT model still provides an indication of growth that can occur.

Once conditions in the egg become suitable for growth, any *Salmonella* present in the egg will grow at a rate dependent on temperature and will rapidly reach levels likely to deliver an infectious dose.

The following table summarises the number of days at given storage temperatures until 20% of eggs contain *Salmonella* at infectious doses.

The values calculated may not be directly applicable to *Salmonella* serovars in New Zealand. However, they do demonstrate a rapid reduction in YMT and increase in growth rate as the storage temperature rises.

Prediction of time until 20% of eggs permit growth of *Salmonella* to levels of concern assuming contamination by a single cell of *Salmonella*

Temperature	Days to YMT*	Days for 1 log growth of *Salmonella***	Days until 20% eggs contain 10 cells	Total days until 20% eggs contain 100 cells
5˚C	74.9	NG	NG	NG
10˚C	45.9	2.0	47.9	49.9
15˚C	28.1	0.5	28.6	29.1
20˚C	17.2	0.2	17.4	17.6
25˚C	10.5	0.1	10.6	10.7
30˚C	6.5	0.1	6.6	6.7

* calculated using YMT equation in Whiting *et al*. (2000)
** calculated using the Rosso model in Thomas *et al*. (2006)
NG = no growth. Growth of *Salmonella* would not be expected at temperatures below 7˚C

On the basis of the information available to date, it would be prudent in New Zealand to maintain the current requirements for handling and storage of eggs.

MPI will maintain a watching brief on the outcomes of EC funded projects currently underway and consider amending the requirement if sound evidence supporting an alternative approach becomes available.

1 Introduction

While eggs are a staple, economical and highly nutritious food, in recent years salmonellosis associated with raw and lightly cooked eggs has become a major public health problem in many countries. Salmonellosis is an acute gastrointestinal disease which commonly lasts for between 3-5 days. Symptoms often include abdominal pain, nausea, diarrhoea, vomiting, fever and chills (Bryan & Doyle, 1995).

A surge in cases of salmonellosis in the US between May and November 2010 resulted in 1939 illnesses over the regular baseline for this period. The Centres for Disease Control reported that these were likely to be part of a *Salmonella* Enteritidis outbreak associated with eggs (Anon., 2010a), and half a billion eggs were recalled as a result (Anon., 2010b).

Eggs are less commonly implicated in salmonellosis in New Zealand[1] and Australia[2] than in other regions of the world, and have traditionally not been considered a particular *Salmonella* risk. It is believed that the key reason for this difference is that the dominant serovars found in flocks in Australia and New Zealand primarily contaminate eggs via faecal material on egg surfaces as opposed to vertical transmission in the ovary. Nevertheless, the incidence of egg-associated salmonellosis has risen rapidly in Australia over the past five years. There is no evidence of such a trend in New Zealand.

In 2004, NZFSA provided a risk management programme template to assist all egg producers to meet the requirements of the Animal Products Act 1999. This template contained agreed storage and shelf-life options based upon then current industry practice (Egg Producers Federation of New Zealand Inc, Code of Practice, 2002) and on the findings of a range of scientific studies. The options for storage time for eggs from the date of lay as specified in the risk management template are:

- 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.

The assumption was that over time and given favourable conditions, any *Salmonella* present on the shell can penetrate the egg and grow to unacceptable levels, and that time/temperature factors can be manipulated to reduce the risk to the consumer.

Concerns have been raised, however, that these assumptions may be flawed and that the requirements may not be locally relevant. Specifically, it has been suggested that;

- There is a lack of evidence of internal contamination of New Zealand eggs with *Salmonella*.
- There is a low incidence of *Salmonella* contamination on the shells of eggs in New Zealand.
- Data from overseas studies based on *Salmonella* Enteritidis may not be relevant to strains found in New Zealand.
- There is minimal evidence of food-borne illness attributable to eggs in New Zealand.

[1] NZFSA-ESR Risk Profile: *Salmonella* (non-typhoidal) in and on eggs (Lake *et al*. 2004).
[2] AEC-SARDI Quantitative Risk Assessment of Eggs and Egg Products (Thomas *et al*., 2006); Public Health and Safety Risk Assessment of Eggs and Egg Products in Australia, (FSANZ, 2009).

- Audits supposedly indicate poor compliance by the retail sector with the required storage conditions.

This project was undertaken at the request of the MPI *Salmonella* Risk Management Strategy Working Group to examine these assertions and to ensure that risk management decisions can be made with reference to relevant and current scientific knowledge.

While eggs from duck, ostrich and quail are consumed in New Zealand, most eggs for human consumption in New Zealand are, and most available information concerns, chicken (*Gallus gallus*) eggs. This assessment report is therefore limited to horizontal transfer and growth of *Salmonella* in chicken eggs.

1.1 RISK MANAGEMENT QUESTIONS

This project examines the above concerns and addresses the following risk management questions for chicken eggs:

1 How likely is it that *Salmonella* contaminating the external surface of freshly laid eggs can penetrate the shell, and how long does it take to occur?

2 Once inside the egg, what is the impact of time and temperature on growth of *Salmonella*?

1.2 ASSESSMENT APPROACH

Presentation of:

- Background information on *Salmonella* and the means by which eggs can become contaminated.
- Data on the prevalence of *Salmonella* on eggs from New Zealand and international surveys.
- Review of the literature on trans-shell transmission.
- A summary of work undertaken to model relationships between temperature, time, and capacity for an egg to support growth and on a model used to predict the rate of growth of *Salmonella* within the egg.
- The implications of the above to the New Zealand situation.
- Recommendations about the appropriateness of New Zealand's current requirements for egg storage and shelf life.

2 General information

2.1 *SALMONELLA*

Salmonella are Gram-negative, non-spore forming, predominantly motile bacteria of the enterobacteriaceae family. *Salmonellae* are grouped into two species *Salmonella enterica* and *Salmonella bongori*. *Salmonella* serovars relevant to food safety most often belong to *S. enterica* therefore only these will be considered in this report. Within the species *S. enterica* there are several subspecies and within those there are many (several hundred) serovars.

Commonly used notation for *Salmonella* serovars is shortened to include the non-italicised serotype name. Therefore *S. enterica* sub species *enterica* serovar Enteritidis becomes *S.* Enteritidis. Various serovars may be identified by detecting O (somatic) and H (flagellar) antigens by agglutination. Further grouping is carried out by phage susceptibility typing (PT) (Jay *et al*., 2003).

All *Salmonella* serovars are considered potential pathogens in humans (Jay *et al*., 2003) although the frequency and severity of illness may vary considerably. Some serovars may commonly cause disease in particular species of animals but rarely cause illness in humans. This report will focus on the serovars that could be transmitted from poultry to humans via contaminated eggs.

S. Enteritidis is the dominant serovar in layer flocks in Europe and the United States. *S.* Enteritidis can be present in a high proportion of the birds in an infected flock and have the capacity to colonise the ovaries and contaminate eggs prior to shell formation. For this reason, most *Salmonella* infections attributable to eggs in these countries are caused by this serovar. In recent years, most international research into *Salmonella* in and on eggs has focussed on *S.* Enteritidis.

However *S.* Enteritidis has not been identified as endemic in New Zealand flocks, and is infrequently detected in Australia. The organism is not believed to be a public health concern in Australia or New Zealand at this time.

The following describes characteristics of *Salmonella* that enable it to potentially survive and grow on and in eggs. *Salmonella* can survive in the environment for months, can grow between 7ºC and 49.5ºC, and under certain conditions such as low water activity (a_w) has been shown to be highly resistant to heat (MPI pathogen data sheet http://www.foodsafety.govt.nz/elibrary/industry/non-typhoid-*Salmonellae*.pdf)

pH values beyond 4.5 and 9.0 are known to have an inactivation effect, although the extent of the kill depends on other intrinsic properties of the food (Jay *et al*., 2003). In the case of eggs, this is important as the albumen pH may rise to 9.1-9.6 as carbon dioxide diffuses out of the egg during the first few hours after lay (Garibaldi, 1960; Board, 1969 [cited in ICMSF, 2000]).

The ability of *Salmonella* to cause illness, reflected in its dose-response, depends on the serotype, host factors and upon the food matrix (http://www.foodsafety.govt.nz/elibrary/industry/non-typhoid-*Salmonellae*.pdf), and, the infectious dose (i.e. minimum number of bacteria that are required to infect but not necessarily cause illness) may be very low in some high fat/low water activity foods (Table 1).

Table 1 Examples of infectious dose for *Salmonella*

Food Vehicle	Serovar	Infective Dose	Reference
Chocolate	*S.* Typhimurium	≤ 10	(Daoust, 1994)
Cheddar Cheese	*S.* Heidelberg	100	(Daoust, 1994)
Cheddar Cheese	*S.* Typhimurium	1-10	(Daoust, 1994)
Ice cream	*S.* Enteritidis	~ 28	(Vought & Tatini, 1998)

Table adapted from (Jay *et al*., 2003).

High protein foods such as boiled egg white have been demonstrated to induce some acid tolerance in *S.* Typhimurium and it has been speculated that this mechanism may provide some protection against stomach acidity, potentially lowering the infectious dose in such matrices (Waterman & Small, 1998).

2.2 CONTAMINATION OF EGGS

Salmonella are transmitted from infected hens to intact eggs either

- vertically, when the egg becomes contaminated in the ovary prior to shell formation ('trans-ovarian transmission'), or
- horizontally, when the outside of the egg becomes contaminated by faecal material and the *Salmonella* enter through the shell.

2.2.1 Vertical (trans-ovarian) transmission

The primary route of transmission of *S.* Enteritidis from hen to egg is trans-ovarian (Messens *et al*, 2005b). There is currently limited information on whether other serovars are capable of trans-ovarian transmission, although serovars including *S.* Typhimurium, *S.* Heidelberg and *S.* Infantis are capable of infecting hens' ovaries (Barnhart *et al*, 1991; Snoeyenbos *et al*, 1969) and there is some evidence that these serovars can be transmitted to eggs via the trans-ovarian route (Poppe *et al*, 1998).

The ability of different *Salmonella* serovars to infect the ovaries of hens has been studied by inoculating layer hens intravenously with six strains of *Salmonella* (Gantois *et al*., 2008), and quantifying bacteria in the ovaries and spleen over time to observe preferences in organ colonisation. One week after inoculation, two *S.* Enteritidis strains and one *S.* Typhimurium strain showed preferential colonisation of the oviduct whereas serotypes *S.* Heidelberg, *S.* Virchow and *S.* Hadar did not. Two weeks after infection, the S. Enteritidis strain S1400 showed significantly higher colonisation of the oviduct than any other strain. Other strains of *S.* Enteritidis were less able to colonize the oviduct and the overall variability within a serotype was similar to that between serotypes.

A wide range of non- Enteritidis *Salmonella* serovars may be present in New Zealand layer flocks. While it is generally assumed that oviduct colonisation does not occur, hard evidence to support this assumption is limited.

2.2.2 Horizontal (trans-shell) transmission

Trans-shell transmission after contamination from the environment or the faeces of infected hens is believed to be the most important route by which egg albumen and yolks become contaminated with serovars other than *S.* Enteritidis (Humphrey, 1994). Generally, the egg structure acts to minimise ingress of contaminating organisms. Penetration of eggs by organisms on their surfaces is addressed in detail in section 4.

It should be noted that even in the absence of transmission of *Salmonella* to the egg contents, surface-contaminated eggs have the potential to cause illness. Contents and any food to which they are added may become contaminated when eggs are cracked for use. Foods containing raw or lightly cooked eggs (e.g. egg nogs, mayonnaise, tiramisu) are more likely to cause illness. Cross contamination may also take place from the shell to foods via hands, utensils and surfaces or directly to the mouth.

2.3 ATTRIBUTION

Salmonellosis is on the list of infectious diseases required to be notified to a Medical Officer of Health and the Local Authority under the New Zealand Health Act 1956. This data is collated in the EpiServ database administered by ESR.

A systematic review and analysis of published and unpublished data sources of the aetiology of salmonellosis in New Zealand undertaken for MPI found that it is very likely (greater than 90% probability) that contaminated food is the cause of greater than 50% of all cases of salmonellosis in New Zealand (Wilson & Baker, 2009), although eggs are likely (greater than 66% probability) to be the cause of less than 10% of cases. This is a much smaller role in the aetiology than is seen internationally (Lake *et al*., 2004).

Eggs were implicated in three of 204 New Zealand outbreaks of salmonellosis between 2000 and 2009 (Table 2). However, the low proportion of outbreaks for which there was strong evidence to identify any source or vehicle, let alone eggs, and the wide variety of suspected foods makes attributing risk very difficult (King & Lake, 2007).

Table 2 Summary of 2000-2009 *Salmonella* outbreaks where eggs were implicated as the source. (Table adapted from King & Lake, 2007).

Setting	Year	Pathogen	Implicated vehicle
Household	2001	*S*. Typhimurium DT160	Raw egg mayonnaise
Food Vendor	2005	*S*. Thompson	Bacon and egg pie*
Event	2001	*S*. Brandenburg	Egg and salmon sandwich

* Multiple food sources listed. All contain poultry products.

Sauces, custards, aoli/mayonnaise, hollandaise and desserts such as homemade ice cream and tiramisu have been associated with outbreaks in other countries. While some of these products do not always contain raw eggs, those that do are not often thought of as 'raw or lightly cooked egg products' by the general public and this "mis-identification" may contribute to failure to identify eggs as a cause of illness particularly in sporadic cases.

2.4 AUSTRALIA OUTBREAKS

A significant increase in non-*S*. Enteritidis outbreaks associated with raw or lightly cooked eggs/egg products began in Australia in 2005 (Table 3) and has not abated (Cameron Moffatt, OzFoodNet, pers. comm. 2010), with a recall in 2011 of eggs implicated in an outbreak of salmonellosis in Queensland[3]. Possible causes include the extension of the shelf life for eggs (6 weeks from the time of packing is the current industry standard), and a change in consumer habits (e.g. a possible increase in consumption of raw/lightly cooked egg sauces and desserts). It is also plausible that staff undertaking investigations are now more likely to identify egg-related cases as there is now a greater awareness of eggs as a possible vehicle for salmonellosis in Australia (Katrina Knope, OzFoodNet, pers. comm., 2010).

Studies have not been undertaken to determine whether outbreak strains can be transmitted vertically. In fact, it is generally assumed that the Australian illnesses have been caused through use of cracked or dirty eggs, introducing *Salmonella* from egg surfaces into food products or by migration of bacteria into the egg contents through the shell.

Table 3 Egg associated outbreaks of Salmonellosis, Australia, 2001-2008 (OzFoodNet)

Year	Foodborne illness outbreaks	Salmonellosis outbreaks	Egg-associated outbreaks
2001	86	19	3
2002	92	26	5
2003	102	31	4
2004	118	36	6
2005	102	33	14
2006	115	41	16
2007	149	50	24
2008	104	35	20

Katrina Knope, OzFoodNet, Dept. Health and Aging, Australia (Pers. Comm.)

The reason salmonellosis attributable to eggs remains low in New Zealand is unknown, but may include:

- Recommended unrefrigerated storage life of 21 days (3 weeks) in New Zealand compared with an industry norm in Australia of a six week shelf-life with a recommended storage temperature of below 15°C ± 3°C; (a shorter time would be expected to minimise both the likelihood of shell penetration and the proportion of eggs in the marketplace that would be capable of supporting the growth *of Salmonella*).

[3] http://www.safefood.qld.gov.au/index.php?option=com_content&view=article&catid=76&id=336&Itemid=36 (accessed March 2011)

Note that the Eggs Producers Federation of New Zealand (EPFNZ) believes that actual practice in New Zealand differs substantially from the recommendations.

- Differences in consumer behaviour, albeit undefined, and failure to recognise raw eggs as ingredients of composite foods during investigations

- Individuals conducting case investigations are less aware of eggs as a potential vector in New Zealand.

3 *Salmonella* on and in eggs

3.1 SURVEYS OF SALMONELLA IN NEW ZEALAND AND INTERNATIONALLY

The findings of New Zealand and international microbiological surveys undertaken to investigate the extent of *Salmonella* contamination on and in eggs at retail are presented in Table 4.

While variation in the sampling plans used has some impact on the reported outcomes of the surveys, the surveys still provide an estimate of contamination levels of non-*S.* Enteritidis on and in eggs; non-*S.* Enteritidis serotypes being of most significance to New Zealand.

Table 4 Extent of contamination by *Salmonella* serotypes on and in eggs at retail in New Zealand and internationally (adapted from Thomas *et al*., 2006)

Country	Study year	Number of samples	Sample	*Salmonella* Enteritidis	Other *Salmonella*	Reference
New Zealand	1994	2037	Shell	0	0	Johnson, 1995
		2046	Contents	0	0	
New Zealand	2007	514	Shell	0	9 (1.8%)	Wilson, 2007
		3710	Contents	0	0[1]	
Northern Ireland	1996-97	12540*	Shell	2*	6* (0.047- 0.29%)[2]	Wilson *et al*., 1998
		12540*	Contents	1*	0	
UK	1995-96	83820*	Shell	103*	17* (0.02-0.12%)	Wall & Ward, 1999
		83820*	Contents	16*	2* (0.024-0.14%)	
Australia	2003	6476	Shell	n/a	0	Thomas *et al*., 2006 (quote SARDI 2003, unpublished)
		20000	Contents	n/a	0	
UK	2003	28488*	Shell	7*	2* (0.007-0.042%)	Food Standards Agency, 2005
		28488*	Contents	0*	0*	

* Tested in groups of six eggs

[1] One egg from each six-pack was tested for surface contamination, and nine eggs of 514 were positive giving a 1.8% prevalence. The remaining 3710 eggs were pooled for contents testing (each result could have represented 6 or 30 eggs).

[2] The methodologies of the 6-pack involved all 6 contents (yolks, etc.) being poured aseptically into a bag and the shells all aseptically crushed together. Although there were 2 positive samples for *S.* Enteriditis isolates in the shell, it could have been anywhere between 2-12 eggs contaminated.

The overall rate of contamination with non-*S.* Enteritidis isolates on the shells of eggs irrespective of the sampling plan used ranged from not detectable to 1.8%[4].

[4] The actual incidence maybe six time less depending on whether 1 or more of the eggs in the six pack was contaminated

To formally compare countries, however, the effect of the different sampling plans was evaluated with respect to the range of possible egg surface prevalence and the confidence intervals around the multiple ranges.

With respect to non-*S.* Enteritidis, both New Zealand studies enriched single eggs and the calculation of prevalence and 95% confidence intervals was straightforward. Non-*S.* Enteritidis species were not detected on the surface of 2037 eggs in the 1994 survey (CI 0% - 0.18%) and nine were detected on 514 eggs (1.75%; CI 0.8% - 3.3%) in the 2007 survey.

Similarly, non-*S.* Enteritidis species were not detected on 6476 individually enriched eggs in the Australia 2003 survey (CI 0% - 0.06%).

In contrast, the UK (1995-1996), Northern Ireland (1996-1997) and UK (2003) surveys pooled six eggs prior to enrichment giving a possible range of egg surface prevalence depending on the number of eggs in the pool that were actually positive. For example, the prevalence calculated if just one egg was positive would be six times greater on a population basis if all six eggs were positive. Similarly, the 95% confidence intervals would vary depending on the number of eggs considered to be positive.

Figure 1 summarises the findings of New Zealand and international microbiological surveys undertaken to investigate the extent of *Salmonella* contamination on eggs at retail taking account of the worst case population prevalence, i.e. individual eggs positive for New Zealand and Australian surveys; all six eggs in a positive pool positive for UK (1995-1996), Northern Ireland (1996-1997) and UK (2003) surveys.

The UK (1995-1996), Northern Ireland (1996-1997) and UK (2003) surveys enriched in groups of six eggs give possible egg surface prevalence ranges of 0.02% - 0.12% (CI 0.01% - 0.15%), 0.05% - 0.29% (CI 0.02% - 0.38%), and 0.007% - 0.04% (CI 0.001% - 0.07%), respectively.

This method of analysis whereby the range of possible prevalence is presented better represents the possible uneven distribution of *Salmonella* contamination in the retail egg population.

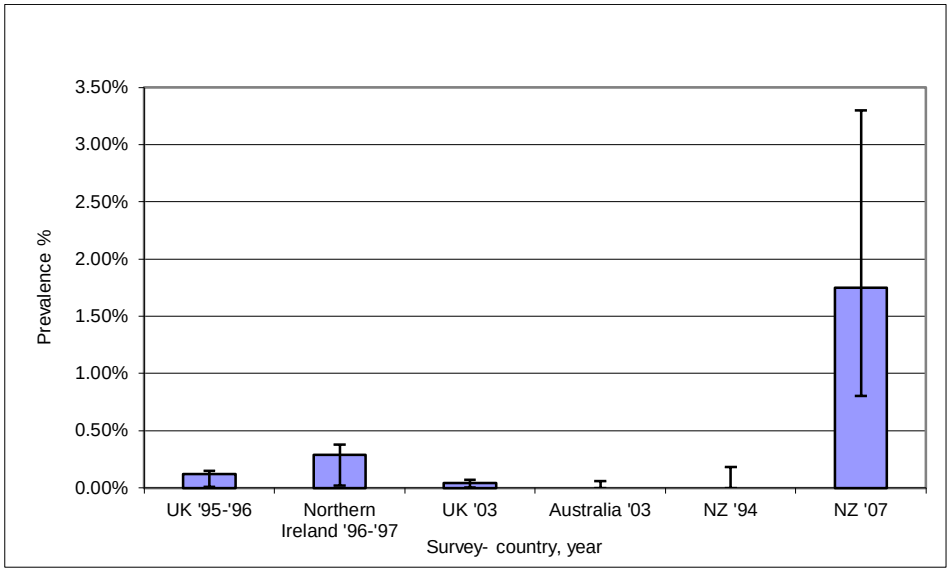
It is obvious, therefore, that the prevalence of non-*S.* Enteritidis *Salmonella* on New Zealand egg shells is substantially higher than has been reported from large surveys undertaken overseas.

Adjusting this rate of contamination for cartons of 12 eggs (most common retail unit in new Zealand), and assuming uniform distribution, approximately one in five (0.017 x 12 = 0.21) cartons of eggs sold in New Zealand at retail would contain on average one egg with surface *Salmonella* contamination. An earlier study (1994) of 2037 shell samples and 2046 contents samples, did not detect *Salmonella* on the shell or in the contents. When the tests results from two studies are combined, the rate of contamination would be on average one egg contaminated in every seven cartons of eggs. In comparison, the rate in the UK, assuming comparability of sampling plans, would be one carton contaminated in every 4166 cartons.

More importantly, spikes of contamination and hence risk, can occur, as observed in a 2001 outbreak investigation in New Zealand (Thornley, 2003) in which a survey of 93 eggs detected *Salmonella* on the shells of 15% (95% CI 8.5-24%) but not in the contents. A reason for this high percentage was not identified, and evidence is not presented to support any

geographic or temporal effects, greater infection within the flock providing the eggs, or the way in which the eggs were handled.

Figure 1 Extent of contamination by non-*S.* Enteritidis serotypes on the surface of eggs at retail in New Zealand and overseas (error bars reflect 95 percent confidence intervals)



The failure to detect internal contamination of eggs in New Zealand is not surprising. Firstly, *S.* Enteritidis is not endemic in our flocks hence contamination of the contents by vertical transmission is unlikely. Secondly, contamination of the content by penetration of the egg shell not only depends on the rate of contamination of the shell but also on the rate of penetration. The rate on the shell is described above as 1.75%. The rate of penetration is unknown.

However, the results of the UK 1995-1996 study (Table 4) suggest that two of 17 eggs contaminated on the shell had contaminated content; a proportion of 12% with a lower 95th confidence interval of 1.5%. The number of eggs required to be sampled to detect internal contamination assuming these rates of penetration (and similar genetic makeup of New Zealand and UK flocks) would be anywhere from 1000-15,000. The sample size in the New Zealand 2007 study might, therefore, have been too small (n=3710), requiring a penetration rate greater than 4.5% to provide a 95% likelihood of a single detection.

4 Penetration of eggs by *Salmonella*

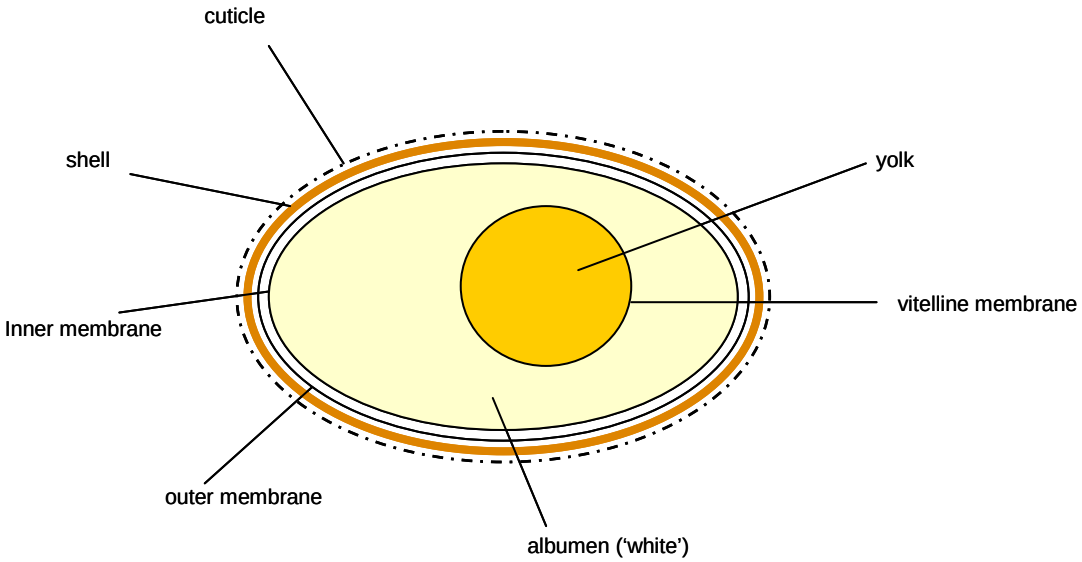
Hens have a single orifice for defecation and reproduction so surface contamination with faecal material, hence faecal pathogens if present, may occur during or after lay. Penetration of the pathogens through the shell may then occur.

The likelihood and rate of penetration of bacteria into the contents is influenced by many factors; some relating to the egg (intrinsic) or others external to the egg (extrinsic). How the eggs are handled or treated through the production chain (extrinsic factors) can affect the integrity of the intrinsic factors that protect the egg from penetration.

4.1 INTRINSIC FACTORS

Intrinsic factors are structural components of the egg that protect the egg from bacterial invasion. The main structures of an egg are the waxy cuticle, the shell, the shell membranes, the white (albumen), the vitelline membrane and the yolk (Figure 2).

Figure 2 Egg structure



4.1.1 Cuticle

The cuticle layer that coats the egg shell is largely made up of proteins and is permeable to gases. It is thought to form the egg's outermost defence against bacterial penetration.

Sparks & Board (1985) used electron microscopy to demonstrate that the cuticle is not fully formed and pores in the shell are open in the first minutes after lay. After this time the cuticle matures and the pores in the shell become sealed. The authors concluded that bacterial defence by the cuticle is an attribute acquired after oviposition, and that eggs are more easily penetrated immediately after lay.

When mature, the cuticle is an effective barrier to water by covering the pores in the egg shell. However, as the egg ages the cuticle becomes less effective, possibly due to cracking (Messens *et al.*, 2005b).
The European Sabre (eggshell quality) project[5] recognised that trans-shell contamination may have a more significant impact on egg safety in the future, and has undertaken work to quantify the cuticle. They established that variation in cuticle quality is correlated with bacterial penetration and also identified that cuticle quality has a genetic component.

In contrast, a number of authors have questioned the importance of the cuticle as a primary defence mechanism. Nascimento *et al.* (1992) observed by scanning electron microscopy that the mature cuticle very rarely covers the whole egg surface regardless of the age of laying hens. They also demonstrated a correlation between pore number and penetration, and, although their results were variable, questioned the cuticle as the first line of defence for eggs.

Similarly, Messens *et al*. (2005a) also studied cuticle quality in eggs from hens of various ages. After inoculating artificially filled eggs with *S*. Enteritidis, the researchers concluded that cuticle quality, as judged visually by staining and quantification by computer software was not correlated with *Salmonella* penetration. However, the time after lay of inoculation was not factored in, so the study is not considered to invalidate the work of Sparks and Board (1985).

Washing of eggs damages the cuticle, and for this reason washed eggs are often oiled to prevent penetration of bacteria. Nonetheless, the cuticle is still likely to be an important barrier to pathogens in unwashed eggs. In New Zealand, some egg producers wash eggs according to agreed criteria (EPFNZ/NZFSA, 2002).

4.1.2 Shell

The effectiveness of the egg shell, mainly composed of calcium carbonate, as a barrier to bacterial penetration of eggs has yet to be defined.

4.1.2.1 Structural integrity

Visible cracks in the egg shell obviously enable pathogens to breach any protective barriers. However, visibly cracked eggs should be identified during the initial candling and sorting of eggs and diverted away from retail to low risk uses (EPFNZ/NZFSA, 2002).

Micro-cracks in egg shells formed during rapid cooling of eggs were visible with scanning electron microscopy (SEM) studies. These present a potential route of penetration through the shell and caused an increase in penetration from 48% to 91%. The study authors concluded that processors would need to find a balance between the rate of cooling and storage time at higher temperatures. Slow cooling is an appropriate procedure as long as it is initiated soon after lay (Fajardo *et al.*, 1995).

The egg shell also has pores that not only allow gas transfer but can allow trans-shell bacterial penetration when the cuticle is not intact (Fajardo *et al*., 1994). The membranes inside the egg

[5] Sabre, a European Sixth Framework Programme, is studying the impacts of alternative practices on egg safety and quality, in particular egg shell formation, egg shell and cuticle quality, and determining genetic markers of egg quality to allow selective breeding to improve egg safety and quality (http://ec.europa.eu/research/agriculture/success_sabre_en.htm).

shell (outer coarse membrane and inner fine membrane) form an additional barrier should the cuticle and shell defences be breached (refer 4.1.3).

4.1.2.2 Shell quality and thickness

The effectiveness of the shell itself as a defence mechanism depends on the quality of the shell (Nascimento *et al*., 1992), which is commonly defined in terms of the quantity of shell present and can be assessed by measuring shell weight, thickness or specific gravity. There are many factors that influence shell quality such as the environmental temperature in which the hens are reared, dietary factors, age of the hens and stress to which they hens are exposed during the laying time.

Maximum egg shell thickness occurs when hens are approximately 42 weeks old (Messens *et al.*, 2005a). As hens age, the eggs they produce increase in size although they contain roughly the same calcium content. Consequently, older hens produce thinner eggs (Messens *et al*,. 2005a). Nascimento *et al*. (1992) observed an inverse correlation between egg shell thickness and penetration of *S*. Enteritidis, neither Messens *et al.* (2005a) using artificially filled eggs challenged by suspension in *S*. Enteritidis or Berrang *et al.* (1999) using a membrane challenge apparatus with *S*. Typhimurium, found any correlation between the age of the hen at lay and penetration through the shell and membrane complex. Berrang *et al*,. (1999) concluded that as the layer hens grew older, the eggs they produced were actually more resistant to penetration.

In general, higher quality eggs have a higher specific gravity. In a large study of 7560 eggs, Sauter & Petersen (1974) found that when eggs were grouped by specific gravity, there was a clear correlation between penetration and specific gravity. Eggs were challenged by dipping warm eggs in a cold bacterial suspension followed by drying and storage, as would be expected during washing in general production. Thin shell eggs with lower specific gravity were more likely to be penetrated by 12 different *Salmonella* serotypes including *S*. Typhimurium. Unfortunately, in contrast, Williams *et al.* (1968) using *S*. Typhimurium did not observe a correlation when shells were grouped into either a ‘thin shell’ or a ‘thick shell’ group, using similar methods of bacterial challenge.

Work undertaken as part of the European/Canadian SABRE and RESCAPE[6] projects to quantify rates of bacterial penetration of eggs reportedly suffered from similar problems with reproducibility and reliability. The effectiveness of the intact egg shell as a barrier to bacterial penetration therefore remains equivocal with previous studies confounded by the multiple factors that impinge on penetration, bacterial growth and ability to detect penetration.

4.1.3 Shell Membranes

There are two membranes in close proximity to the inside of the egg shell; the outer coarse membrane and the inner fine membrane. The outer coarse membrane does little to protect the egg from penetration but the inner fine membrane is known to trap bacteria and delay their entry into the albumen.

[6] RESCAPE is a collaboration between research groups in Europe and Canada to reduce the level of contamination of eggs from pest and infections under alternative housing regimes through optimising hen production, egg sorting and decontamination methods, and, in the longer-term genetically improving laying hens to resist infection.

Miyamoto *et al*. (1998) dipped eggs of between 0.25 hours and 7 days post lay in suspensions of *S*. Enteritidis and *S*. Typhimurium and held at 25ºC for 10 minutes. The shell and contents were separated aseptically after surface alcohol-flaming sterilisation, and tested for the presence of *Salmonella*. Both *Salmonella*e serovars were detected on the inner egg shell at a rate between 2-8 times that of the egg content, leading to the conclusion that the majority of bacteria were trapped in the egg shell membranes.

Unfortunately, Himathongkham *et al*. (1999) demonstrated that surface decontamination using alcohol-flaming was incomplete and concluded results could falsely indicate contamination of the inner surface. However, Thompson *et al*. (2000) using an improved method of aseptically removing the egg contents still reported a high rate of recovery of *S*. Enteritidis from cultured eggshell and membrane whereas none of the egg contents were contaminated.
It therefore appears that, when intact, the inner membranes form an effective barrier to penetration by bacterial pathogens contaminating the egg shell surface.

4.1.4 Albumen

Should the physical shell and shell associated barriers be breached, the albumen itself forms a chemical defence mechanism preventing growth. Enzymes including lysozyme and conalbumen degrade bacterial cell walls killing the invading bacteria, and sequester metal ions respectively, slowing bacterial growth (Lake *et al*., 2004).

4.1.5 Vitelline Membrane

The vitelline membrane forms a barrier between the chemically active albumen and the nutrient rich yolk.

Cogan *et al*. (2004) suggest that the expression of curli (SEF17) pili (fimbriae) may be important for invasion of the yolk, although the mechanism is unknown. The mechanism may be as simple as fimbriae-mediated attachment to the albumin side of the vitelline membrane, assisted by flagellated motile strains being able to swim across to the membrane through the viscous albumen, and entry to the yolk as the membrane degrades (refer section 5). *S.* Typhimurium in egg albumen invaded the yolk more efficiently than *S*. Enteritidis (Cogan *et al*., 2004).

These observations suggest that once inside the egg, some serovars may be more likely to invade the yolk early and grow to high numbers.

4.2 EXTRINSIC FACTORS

The EPFNZ Code of Practice provides a set of guidelines if commercial eggs for retail are to be washed (EPFNZ/NZFSA, 2002). These guidelines were derived from Stadelman *et al*. (1994), as cited by ICMSF (2000) who provide a list of factors known to affect microbial penetration. Most New Zealand egg producers now follow a Risk Management Programme template that contains washing guidelines also based on this paper. Factors that increase microbial penetration listed in Stadelman *et al*. (1994), include:

- Temperature differential; washing eggs in a liquid of a cooler temperature than that of the egg
- Moisture; processes that wet the shell such as sweating and cleaning with wet cloth
- Faecal matter; visible dirt or faecal matter on the shell and microbial load of wash water
- Washing techniques that damage the cuticle
- Wash water containing iron.

In addition, characteristics of the contaminating pathogen itself including its prevalence and numbers can affect microbial penetration. The relative importance of a number of these factors in determining *Salmonella* penetration is unclear.

4.2.1 Temperature

4.2.1.1 Temperature differential

It is well established that penetration of *Salmonella* into the egg is aided by a positive temperature differential between the egg and the bacterial suspension (Haines & Moran, 1940). Washing warm eggs in cool water, immediately after lay or after 1-24 hours of pre-cooling (Haines & Moran, 1940), results in contraction of the egg contents, causes a negative pressure gradient, and bacteria on the surface are sucked into the egg. Both *S.* Enteritidis and *S.* Typhimurium readily penetrate egg shells (0.25 hr – 7days post lay) when the eggs are abruptly exposed to low temperatures, but penetration can be reduced by pre-cooling the eggs (Miyamoto *et al.*, 1998).

4.2.1.2 Cooling

Eggs subjected to rapid cooling were more prone to penetration by *S.* Enteritidis but only when returned to and stored at room temperature (Fajardo *et al.*, 1995). The increased rate of penetration was thought due to microscopic cracks as a result of cooling (Fajardo *et al.*, 1995). Rapid cooling did not lead to increased penetration of *S.* Enteritidis if the eggs were maintained at ~7°C after rapid cooling (Chen *et al.*, 2002a; Chen *et al.*, 2002b), however strain/serovar effects were not examined.

4.2.2 Prevalence on the shell

The effect of the concentration of *Salmonella* on the surface of the egg on penetration for both *S.* Enteritidis and *S.* Typhimurium was studied by suspending eggs in either low (10^3) or high (10^6) concentrations of *Salmonellae* (Miyamoto *et al.*, 1998). *Salmonella* was detected inside the shell at consistently higher rates in the high inoculum groups than in the low inoculum groups for both *Salmonellae* serovars, although the methods employed to surface sterilize the eggs prior to sampling have again been questioned (Himathongkham *et al.*, 1999).

4.2.3 Moisture

When eggs are placed at room temperature following refrigerated storage, condensation often forms on the egg shell (sweating). Fromm and Margolf (1958) showed that the rate of penetration by *Salmonella* was higher if eggs were allowed to sweat for one hour and

particularly likely after three or five hours sweating, although Ernst *et al.* (1998) were not able to demonstrate any effect of sweating on penetration by *S.* Enteritidis.

Williams *et al*. (1968) examined the effect of moisture more directly by experimentally varying moisture content of previously dried faecal samples placed on the surface of eggs. They found that reconstituted faecal material of a consistency equivalent to that of avian faeces (Williams & Whittemore, 1967) was optimal for penetration and that moisture levels less than or greater than normal resulted in reduced penetration; the latter suggested by the authors to be a possible artefact.

Padron (1990) found that inoculation by spraying soluble bacterial solution directly onto the eggshell enhanced the ability to *S.* Typhimurium to penetrate eggs, but inoculation by contact with contaminated litter with no additional water lessened penetration.

4.2.4 Serovar and motility

While penetration of eggs is not dependant on the serotype of *Salmonella* (Williams *et al*., 1968; Schoeni, *et al.*, 1995) or the motility of strains (Williams *et al*., 1968), the presence of flagella, hence motility was shown to reduce the time in which penetration occurred (Williams *et al*., 1968).

As a consequence, experiments to investigate the effects of various extrinsic factors on penetration may be confounded by the period between exposure to the pathogen and measurement of penetration. A short period might only show penetration with motile strains whereas a long period could show penetration with both motile and non-motile strains, should penetration be able to occur.

4.3 SUMMARY

The penetration of *Salmonella* through the shell and into the contents of eggs involves a number of intrinsic and extrinsic factors. The integrity of the cuticle, shell and the shell membranes is the most important defence mechanism; damage to any probably allowing penetration by pathogens contaminating the outside surface of the egg.

Extrinsic factors such as the temperature of the egg (rate of cooling, differential with environment and storage), surface moisture level and degree of contamination of the shell surface appear to be more important to penetration of *Salmonella* than bacterial serovar. The relative importance of each of these factors is currently difficult to ascertain.

There have been questions raised over the methods used for investigation of bacterial penetration, and consequently results of studies have been conflicting. Similarly, interpretation of results has been difficult due to confounding factors in the experimental design.

It is hoped that projects associated with the European/Canadian SABRE and RESCAPE programmes design better methods to detect and quantify bacterial penetration of egg shells, allow better definition of bacterial penetration and the factors that affect penetration, and ultimately provide recommendations for control of penetration.

Nevertheless, it is possible that penetration of the shell can occur under commercial conditions, and that some processing conditions make penetration more likely. Penetration of *S.* Enteriditis and *S.* Typhimuium was similar. While the numbers of bacteria penetrating the shell may be low, subsequent environmental conditions must be such that further growth is prevented, or at the very least minimised.

5 Growth in the egg

A number of albumen components limit the growth of bacteria that breach the shell and outer membrane of the egg. Enzymes including lysozyme and conalbumen degrade bacterial cell walls and sequester metal ions respectively, slowing bacterial growth (Lake *et al.*, 2004). Carbon dioxide diffuses out of the egg during the first few hours of lay, causing the albumen pH rises to rise to 9.1-9.6 [adapted from Garabaldi, 1960, Board, 1969, cited in ICMSF, 2000], which would also inhibit bacterial growth.

The vitelline membrane surrounds the yolk. The yolk readily supports bacterial growth. The vitelline membrane prevents yolk components leaking into the albumen (Humphrey, 1994).

Salmonella growth in the albumen is suppressed by a variety of factors (Humphrey, 1994). However, at the right temperature, the yolk can support rapid growth to high numbers (Braun & Fehlhaber, 1995). Whiting *et al*. (2000) have proposed that vitelline membrane integrity is critical in the control of growth of *Salmonellae* in eggs. Either the vitelline membrane prevents microbial penetration, or it prevents leakage of non-visible yolk fractions into the surrounding albumen that would otherwise support microbial growth. This proposal was based on observations that naturally infected eggs stored at 20ºC did not support growth of *S.* Enteritidis to levels above 20 CFU/egg *(*Humphrey *et al.*, 1991). It is thought that the vitelline membrane integrity is disrupted in a temperature/time dependent manner.

Salmonella growth in eggs has been modelled for the purposes of risk assessment. The model employed by Thomas *et al*. (2006) for the SARDI risk assessment is likely to be the most relevant to New Zealand as it considers the limited information available on non- *S.* Enteritidis serovars and assumes that contamination occurs at the time of lay, not before (i.e. it does not accommodate vertical transmission).

This model incorporates two components: initiation of growth, and rate of growth.

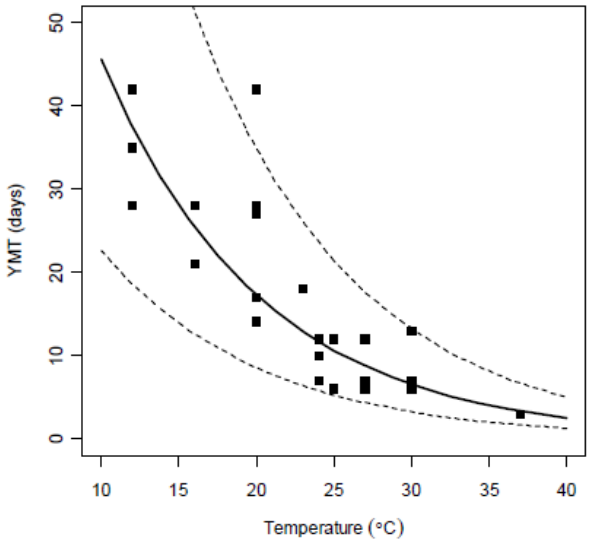
5.1 INITIATION OF GROWTH: THE YOLK MEAN TIME MODEL

The Yolk Mean Time (YMT) is a convenient tool used in several risk assessments (USDA-FSIS (1998), FAO-WHO (2002), and Thomas *et al*. (2006)) to aid in assessing the risk of *Salmonella* from consuming eggs. It reflects the fact that storage temperature affects factors including the integrity of the vitelline membrane and that these factors alter capacity for an egg to support growth of *Salmonella*.

The approach is probability-based, and has been used to determine the number of days at a given temperature, the YMT, before 20% of eggs can support growth of *Salmonella* in the albumen (Whiting *et al*., 2000). Because this approach essentially provides a 'population-level' prediction of YMT, the actual time taken for the conditions in an individual egg to support growth at a given temperature may vary substantially.

The effect of temperature on the YMT can be seen in Figure 3.

Figure 3 YMT versus storage temperature. The solid line shows the fitted model with 95% confidence intervals (dashed lines) with data from Whiting *et al*., (2000) (reproduced from Thomas *et al*., 2006).



The Whiting model describing the effect of temperature on YMT takes the form

$$Log_{10} = 2.0872 - 04257T$$

Where YMT is in days; and T is in °C.

The YMT model had residual standard error of 0.1524 on 31 degrees of freedom, multiple R-squared or 0.7769 and adjusted R-squared of 0.7697

Experimental work investigating temperature-dependent impacts on intrinsic defences of eggs including the vitelline membrane is necessary to contribute to a mechanistic model of time taken for the egg to become a permissive environment for *Salmonella* serovars.

The YMT model does not account for any effect of serovar on initiation of growth. Most YMT investigations have been based on *S*. Enteritidis, however other serovars are more likely to be of concern in New Zealand.

The SARDI report (Thomas *et al*., 2006) describes a study (Cogan *et al*., 2004) in which the albumen of whole eggs were inoculated with low numbers (2-3 cells) of various *Salmonella* strains and found that growth of both *S*. Enteritidis and *S*. Typhimurium occurred in approximately 25% of inoculated eggs in eight days at 20°C whereas neither *S*. Pullorum nor *S*. Gallinarum grew.

While initiation of growth of *S*. Typhimurium is within the confidence intervals of the model, this study shows that the Whiting YMT model is not equally applicable to all serovars and that the initiation of growth for some serotypes occurs faster than predicted and these strains then can grow extremely quickly once growth is initiated (refer section 5.2). This means that there is a possibility that the YMT model may overestimate the time to growth for serovars of concern in New Zealand, and as a consequence underestimate the risk to human health.

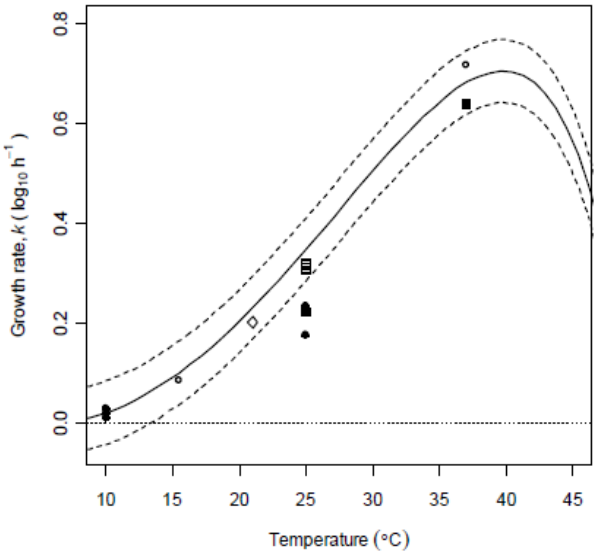
More data is needed to ascertain whether this observation of early onset of growth at room temperature is truly reflective of non- *S.* Enteritidis serovars, including *S.* Typhimurium.

5.2 RATE OF GROWTH

Once the albumen can support bacterial growth, any *Salmonella* present will proliferate at a rate governed by temperature.

While there is a paucity of data on growth of non-*S.* Enteritidis serotypes in eggs (Braun & Fehlhaber, 1995; Cogan *et al.*, 2001; Humphrey, 1994), growth data for *S.* Typhimurium in eggs (Schoeni *et al*, 1994) fits with a model (Thomas *et al.*, 2006) generated for growth of *S.* Typhimurium on chicken meat (Figure 4).

Figure 4 Comparison of growth rates for *S.* Typhimurium in whole egg with the prediction of the Rosso equation for growth of *S.*Typhimurium on chicken meat (solid line) with 95% CI (dashed lines). (Reproduced from: Thomas et al., 2006)[7].



The Rosso model for growth of *Salmonella* on chicken meat may therefore be suitable for estimating growth in egg content once growth is supported (i.e. after YMT has been met), although validation for a wider range of different serotypes may provide a better understanding of its representativeness.

The Rosso model describing the growth of *Salmonella* on chicken meat the takes the form:

$$k = k_{opt} \frac{(T-T_{max})(T-T_{min})^2}{(T_{opt}-T_{min})[(T_{opt}-T_{min})(T-T_{opt}) - (T_{opt}-T_{max})(T_{opt}+T_{min}-2T)]}$$

[7] Data from Bradshaw et al (1990), Gast and Holt (2000, 2001), Ruzickova (1994) and Schoeni et al (1995).

Where k is the growth rate ($log_{10}h^{-1}$) at temperature T (°C), k_{opt} is the optimum growth rate, and T_{min}, T_{opt} and T_{max} are the predicted minimum, optimum and maximum temperatures of growth respectively. The results of the regression are presented in Table 5.

Table 5 Predicted values for the growth rate of *Salmonella* Typhimirium on chicken meat using the Rosso equation

Parameter	Value	Units
k_{opt}	0.7039	$log_{10}h^{-1}$
T_{min}	5.567	°C
T_{opt}	39.76	°C
T_{max}	49.59	°C

As noted previously, Cogan *et al*. (2004) found that, in at least some eggs, growth of both *S*. Enteritidis and *S*. Typhimurium reached high levels (10^6 CFU/ml) in eight days at 20°C whereas neither *S*. Pullorum nor *S*. Gallinarum grew.

Cogan *et al*. (2004) also found that the non-motile strains that did not express flagella were unable to multiply in eggs. The expression of curli (SEF17) pili during either exponential or early stationary phase growth was closely linked to the proportion of eggs in which growth was detected for each strain, regardless of subtypes and serovar. The authors concluded that expression of curli type fimbriae may be important for growth to high levels but the mechanism is unknown.

5.3 SUMMARY

The vitelline membrane is an important structure that prevents the growth of *Salmonella* in the egg albumen by excluding nutrients in the yolk.

Should the defences be breached, growth of *Salmonella* will not occur until the vitelline membrane degrades and conditions become permissive; the time for such being defined as the "yolk mean time (YMT). YMT is temperature dependant: the higher the storage temperature, the shorter the delay until growth can occur. Once the egg can support growth, any *Salmonella* present will grow to high numbers at a rate dependent upon storage temperature.

Models that predict the time/temperature dependent initiation of growth and the growth rate of *Salmonella* should conditions favour growth are available (Thomas *et al*., 2006). While most available data on *Salmonella* in eggs is for *S*. Enteritidis, limited data for *S*. Typhimurium data suggests that these models are useful for non-*S*. Enteritidis serovars, and that models for growth of *Salmonella* on chicken meat appear to be useful for predicting growth in eggs.

Table 6 summarises YMT model and growth predictions at various temperatures until 20% of eggs permit growth of *Salmonella* to levels of concern. The values have been calculated using the Whiting model to calculate the YMT and the Rosso model to calculate days until 1 log growth of *Salmonella* in the egg content once growth is supported.

Table 6 Predictions of time until 20% of eggs permit growth of *Salmonella* to levels of concern assuming contamination by a single cell of *Salmonella* (see Table 1)

Temperature	Days to YMT*	Days for 1 log growth of *Salmonella***	Days until 20% eggs contain 10 cells	Total days until 20% eggs contain 100 cells
5˚C	74.9	NG	NG	NG
10˚C	45.9	2.0	47.9	49.9
15˚C	28.1	0.5	28.6	29.1
20˚C	17.2	0.2	17.4	17.6
25˚C	10.5	0.1	10.6	10.7
30˚C	6.5	0.1	6.6	6.7

* calculated using YMT equation in Whiting *et al*. (2000)
** calculated using the Rosso model in Thomas *et al*. (2006)
NG = no growth. Growth of *Salmonella* would not be expected at temperatures below 7˚C

Values have been calculated for time until 20% of eggs contain 10 or 100 cells as this relates to the infective dose (see Table 1).

The table demonstrates that once conditions in the egg become suitable for growth, and for example at 20˚C, 20% of eggs may be suitable for growth in only 17.2 days, any *Salmonella* present in the egg will grow at a rate dependent on temperature and will rapidly reach levels likely to deliver an infectious dose.

At 15°C the model predicts that it could take ~29 days for 20% of the eggs to allow a single *Salmonella* cell to grow to levels at which human illness is likely to occur. The New Zealand risk management template allows 35 days at 15°C and the Australian guidelines 42 days. At 20°C and 25°C, growth to unacceptable levels would occur in just 18 and 11 days, respectively.

There remains a need to better evaluate the degree of variation in time to growth (YMT). However the time taken for *Salmonella* to reach levels of concern within eggs is small in comparison to the YMT (for all temperatures above 7˚C), deeming investigation of the influence of serovar on growth rate a lower priority.

6 Discussion

Eggs have not been identified as a significant source of human salmonellosis in New Zealand.

A key difference between New Zealand and Europe and the United States where eggs are frequently associated with foodborne illness outbreaks is the absence in New Zealand flocks of specific *S*. Enteritidis subtypes that colonize hen ovaries and are transmitted to eggs via the trans-ovarian route (vertical transfer). In the literature examined, vertical transfer has not been conclusively demonstrated for other serovars of *Salmonella* including serovars present in New Zealand layer flocks.

Although New Zealand surveys have not provided evidence of internal contamination of eggs with *Salmonella*, this may be an artefact of sample size. There remains a possibility that penetration (horizontal transfer) through the shell resulting from a combination of external contamination and storage and handling conditions may occur.

The incidence of egg-associated salmonellosis has risen rapidly in Australia over the past five years. However there is no evidence of such a trend in New Zealand. The reason for the Australian increase is not clear. Poultry management and egg handling practices may offer some explanation for the rates not increasing in New Zealand.

New Zealand's industry is substantially smaller than that in Australia and tends to be vertically integrated. Grandparent stock is supplied from just two breeders and most feed is similarly sourced. Feed is frequently tested for *Salmonella* and if positive, either reprocessed or treated with anti-*Salmonella* agents, e.g. SalCurb. Similarly, breeding flocks are frequently tested and mitigation actions taken if they test positive, however this is not necessarily the case for laying flocks. New Zealand's commercial egg producers have been required to have a registered risk management programme to manage hazards and other risk factors since 2004.

Interestingly the results of surveys, albeit statistically poor in power due to low sample number, show that the rate of contamination of the outside of eggs in New Zealand is substantially greater than that in Australia (Wilson, 2007).

Penetration of the shell is more likely if *Salmonella* is present on more eggs in the population (Miyamoto *et al.*, 1998), and individual eggs are contaminated with greater numbers of *Salmonella*.

Despite this, *Salmonella* has never been detected on the inside of eggs in New Zealand suggesting that subsequent handling may prevent penetration of *Salmonella*, and/or growth in, eggs.

Eggs produced in Australia are currently stored up to six weeks (42 days) after packing, even at temperatures where *Salmonella* would grow well, whereas New Zealand specifies a maximum of three weeks (21 days) where the storage/holding temperature may exceed 15˚C and only permits a storage time of 35 days where the temperature is maintained at less than 15°C.

If New Zealand were to adopt a similar approach to Australian practice, allowing a 35 day storage period irrespective of storage temperature, would the number of foodborne illnesses attributed to eggs increase?

Currently, there is insufficient data for non-*S*. Enteritidis to enable a quantitative risk assessment to be carried out to adequately compare risk estimates for the different regimes. Nevertheless, there is sufficient scientific evidence to suggest that contamination levels in eggs will increase if eggs are stored at >15°C for more than 21 days.

Many studies have demonstrated the capacity of bacteria to breach the intrinsic (cuticle integrity, shell quality, membrane integrity) defences of eggs. Visibly cracked eggs are redirected to risk-reducing processes (e.g. cooking) prior to retail. The likelihood of penetration of supposedly intact eggs has been shown to be affected by a range of extrinsic factors such as hen age, egg age, and temperature, although there is currently insufficient data to clearly identify factors that enhance or lessen the effects on penetration of these factors.

Studies have shown that the protective cuticle is not mature at the time of lay and may not afford full protection immediately after lay (Messens *et al.*, 2005b). The effectiveness of the intact egg shell as a barrier to bacterial penetration remains equivocal with previous studies confounded by the multiple factors that impinge on penetration, bacterial growth and ability to detect penetration. Shell quality may be impacted by genetic factors, the age of the hen and temperature, making it a less effective barrier.

Penetration of eggs is not dependant on the serotype of *Salmonella* (Williams *et al.*, 1968; Schoeni *et al.*, 1995), suggesting that the factors that influence the likelihood of egg penetration are more likely to be egg-related than serovar-related.

The time taken for *Salmonella* to penetrate the surface of an egg is affected by a range and combination of intrinsic and extrinsic factors. Experiments have shown that penetration can be very rapid when eggs are wet, particularly when there is a temperature gradient. Attempts to lessen the rate of contamination of the outside of eggs through washing to remove faecal matter may therefore unwittingly enhance penetration.

While motility of strains is not required for penetration (Williams *et al.*, 1968), the presence of flagella, hence motility, reduces the time to which penetration occurs (Williams *et al.*, 1968). After laying, initial penetration will occur rapidly with motile strains whereas non-motile strains will penetrate as storage time lengthens.

It is therefore possible that penetration of the shell can occur under commercial conditions, and that some processing conditions, currently undefined, make penetration more likely. It is hoped that projects associated with the European/Canadian SABRE and RESCAPE programmes will design better methods to detect and quantify bacterial penetration of egg shells, allow better definition of bacterial penetration and the factors that affect penetration, and ultimately provide recommendations for control of penetration.

Notwithstanding the likelihood of penetration and that the numbers of *Salmonella* penetrating the shell may be low, subsequent environmental conditions must be such that further growth is prevented, or at the very least minimised. *Salmonella* will not grow in albumen and are separated from the nutritious yolk by the vitelline membrane and protective enzymes.

However, the vitelline membrane breaks down with time and the inside of the egg becomes a favourable environment for growth by any *Salmonella* that do penetrate. Membrane breakdown is temperature dependant; the higher the temperature, the shorter the time. Once breakdown commences, any invading *Salmonella* will grow, again at a rate dependant on the temperature of storage.

The YMT model is used to generate the time at a given storage temperature until growth occurs in 20% of eggs. However, the YMT is not an absolute number and time for an individual egg to support growth at a given temperature could vary substantially. The model appears not to apply equally to all serovars and the initiation of growth for some serotypes occurs faster than predicted (Cogan *et al.*, 2004), thereby potentially underestimating the risk to human health of eggs contaminated with these serotypes.

Growth data for non-*S.* Enteritidis in eggs is limited.

Nevertheless, one data set for *S.* Typhimurium in eggs (Schoeni *et al*, 1994) fits a model generated for growth of *S.* Typhimurium in chicken meat (Thomas *et al.*, 2006). Given the rich nutrient environment of an egg, the chicken model should be suitable for estimating growth in egg content once growth is supported, although validation for a wider range of different serotypes is necessary.

Table 6 provides a summary of relevant predictions based on the above models.

7 Conclusion

While there is no evidence that eggs are causing a significant contribution to human foodborne illness in New Zealand, the comparatively high level of contamination on the outside of eggs compared with Australia indicates that it is important to maintain food safety controls during processing and subsequent storage and handling.

The egg itself possesses several defence mechanisms that reduce the likelihood of penetration of *Salmonella* from the outside into the egg. However, studies defining their effectiveness and, more importantly, which factors need to be controlled to prevent penetration during processing and storage/handling are equivocal. Assurances of safety of eggs cannot, therefore, rely on these defence mechanisms and processing factors.

Should the defences be breached, yolk mean time (YMT) defines how long eggs can be stored at a given temperature before the conditions inside 20% of those eggs would permit the growth of *Salmonella*. There is evidence that growth of some non- *S.* Enteritidis serovars may be supported much more quickly than the YMT, indicating that this model may not be equally applicable to all serovars. Validation of the YMT model has been very limited in non-*S.* Enteritidis serovars, and therefore may produce 'fail dangerous' predictions for strains of *Salmonella* contaminating eggs in New Zealand.

Once conditions in the egg become suitable for growth (and at 20˚C 20% of eggs may be suitable for growth in only 17.2 days), any *Salmonella* present in the egg will grow at a rate dependent on temperature and will rapidly reach levels likely to deliver an infectious dose.

Given the above information it would appear prudent to maintain the current requirements for handling and storage of eggs.

MPI will maintain a watching brief on the outcomes of EC funded projects currently underway and will consider amending the Standard if evidence supporting an alternative approach becomes available.

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