AUSTRALIAN EGG CORPORATION LIMITED

Through-Chain *Salmonella* Risk Identification

Final Project Report

A report for the Australian Egg Corporation Limited

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Foreword

A major reputational and public health issue for the egg industry is the presence of *Salmonella* spp. (particularly some Typhimurium serotypes), which can cause salmonellosis in humans, throughout the supply chain. The presence and spread of *Salmonella* depends on numerous variables so there is no single effective control measure. Further, as no one business through-chain operates similarly, the identification, level and management of risks will vary, which means that there is no one 'best practice'. It is more a case of identified 'appropriate practice'. These issues make the control of *Salmonella* a complex issue subject to a combination of both real and perceived risks.

Federal food safety requirements (implemented by the States and Territories) require all food businesses and primary producers to identify all their potential food safety hazards and implement appropriate control measures to address these hazards. This final report for the first 12 months of the AECL *Salmonella* Initiative constitutes a series of risk identification tables to assist all through-chain stakeholders to initiate identification of their potential hazards. The preliminary minimum considerations for risk management provided are designed to stimulate stakeholder discussion so firm conclusions can be made about what constitutes a minimum risk management consideration.

This project was funded from industry revenue, which is matched by funds provided by the Australian Government.

This report is an addition to AECL's range of peer reviewed research publications and an output of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

Most of our publications are available for viewing or downloading through our website:

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Printed copies of this report are available for a nominal postage and handling fee and can be requested by phoning (02) 9409 6999 or emailing research@aecl.org.

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The authors would like to sincerely thank all of those who have contributed to the *Salmonella* Initiative since September 2014. Those contributions have driven the format, focus and contents of this report and established momentum and collaboration that previously did not exist to more effectively address the concern of all through-chain stakeholders – i.e. the rate of human illness linked to the consumption of egg products.

About the Authors

Dr Kylie Hewson has a degree in Biotechnology from Queensland University of Technology and a PhD in Molecular Virology from the University of Melbourne. Kylie had extensive experience with molecular techniques and diagnostic assay development, specifically for viruses and other pathogens that infect poultry, and had more than eight years' experience working in the poultry industry when she came to the AECL as R&D project manager on a one-year contract. This position is part funded through the Poultry CRC industrial internship program. Her project at AECL focussed on through-chain *Salmonella* risk identification and management for eggs.

Dr Raymond Chia was appointed R&D project manager of AECL in October 2015. He holds degrees from National University of Singapore, the University of Queensland and the University of Tasmania, which awarded his PhD in 2010 for his thesis on *Salmonella*. His career prior to joining AECL included more than five years as a QA Manager in a major meat processing company, and work in the chicken meat industry for two large companies in QA roles.

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Abbreviations

AECL	Australian Egg Corporation Limited
CFU	Colony Forming Units
FSANZ	Food Standards Australia and New Zealand
hr	Hour
IP	Intellectual Property
SOP	Standard Operating Procedure
QA	Quality Assurance
Spp.	Species
UK	United Kingdom
USA	United States of America
US FDA	United States Food and Drug Administration
UTAS	University of Tasmania
UV	Ultraviolet
WHO	World Health Organisation
YMT	Yolk Mean Time

Executive Summary

A reputational and public health issue for the egg industry is the presence of *Salmonella* spp. (particularly some Typhimurium serotypes), which can cause salmonellosis in humans, throughout the supply chain. The presence and spread of *Salmonella* depends on numerous variables so there is no single effective control measure. Further, as no one business through-chain operates similarly, the identification, level and management of risks will vary which means that there is not one 'best practice'. Businesses in the supply chain must identify their own 'appropriate' best practice. These issues make the control of *Salmonella* a complex issue subject to a combination of both real and perceived risks.

Salmonella is an organism that is ubiquitous in the environment as well as across a number of hosts (the type of *Salmonella* will depend on which host is affected). To date, there have been over 2500 types of *Salmonella* identified. Not all of these can be found on poultry farms and not all types found on poultry farms are pathogenic to humans.

It is plausible to conclude that the more intense the scrutiny and surveillance at any single stage through-chain, the more likely a type of *Salmonella* will be found. However, there are a few risks (such as the presence of moisture and yolk) that can substantially increase the risk of humans becoming ill from foods prepared with eggs. Fortunately there are also simple interventions available to address these risks. The issue is to ensure that each stakeholder is able to identify their risks and manage them appropriately. Identification and management of *Salmonella* within every stakeholder through-chain is essential for all stakeholders to be able to identify and manage their food safety hazards. This is a requirement under the FSANZ Standards:

For food businesses, FSANZ Standard 3.2.1 clause 3 – "*General food safety program requirements. A food business must* –

> *(a)* ***systematically examine all of its food handling operations in order to identify the potential hazards that may reasonably be expected to occur****;*
>
> *(b) if one or more hazards are identified in accordance with paragraph (a), develop and implement a food safety program to control the hazard or hazards;*
>
> *(c) set out the food safety program in a written document and retain that document at the food premises;*
>
> *(d) comply with the food safety program; and*
>
> *(e) conduct a review of the food safety program at least annually to ensure its adequacy."*

For primary egg producers, FSANZ Standard 4.2.5; Division 2; clause 3 "*General food safety management.*

> *(1)* ***An egg producer must systematically examine all of its production operations to identify potential hazards and implement control measures to address those hazards****.*
>
> *(2) An egg producer must also have evidence to show that a systematic examination has been undertaken and that control measures for those identified hazards have been implemented.*
>
> *(3) An egg producer must operate according to a food safety management statement that sets out how the requirements of this Division are to be or are being complied with."*

However, given the complexity of *Salmonella* it would be incredibly difficult for any stakeholder to identify all potential hazards unless they had a sound working knowledge of *Salmonella*. The AECL *Salmonella* Initiative has identified that efforts need to be made through-chain to ensure that all stakeholders can better identify, and therefore better manage, their *Salmonella* risks to reduce the incidence of human salmonellosis linked to the consumption of egg products.

The safest eggs will be those that are removed from the hens and the environment as quickly as possible, assessed for cracks and visible organic matter, transported and stored in a manner that does not promote bacterial growth, and used in the preparation of food products with high standards of food handling practices to limit cross-contamination of any *Salmonella* present on other foods into the egg food, or from the eggshell into the egg food.

The AECL *Salmonella* Initiative has engaged with numerous key stakeholders, including state regulators/health departments, industry associations, egg producers, researchers and technical consultants (including veterinarians). Given the extensive number of stakeholders, it is not possible to reach them all directly within a 12-month period. Therefore, this consultation process needs to be continued until all stakeholders are able to identify their relevant *Salmonella* risks and implement appropriate controls and interventions.

This report should be used to initiate discussion between through-chain stakeholders to determine what constitutes appropriate management of *Salmonella* at each stage through-chain. The minimum considerations listed in the tables in this report have been developed with relevant stakeholder input, however, they should not be considered the final version as further consultation with key industry stakeholders is required. The risks identified in this report are merely the starting point for each relevant stakeholder's consideration. The aim of the 'why it is a risk, how to control it and other considerations' column in the tables is to improve each stakeholder's understanding of what constitutes a *Salmonella* risk, and how to identify all risks at each stage beyond what is mentioned here.

The aim of the first 12 months of the *Salmonella* Initiative was to coordinate, review, identify and disseminate comprehensive coherent information regarding *Salmonella* and *Salmonella* risk identification and management through-chain. It is not feasible to expect that a raw animal product that comes out of the hen through the same opening as the faecal matter could ever be sterile, so the risk of *Salmonella* will always be present. Therefore, the ultimate aim is to reduce *Salmonella* load through-chain.

1 Introduction

An enduring challenge for eggs is the presence of *Salmonella* spp. (particularly some Typhimurium serotypes), which can cause salmonellosis in humans, throughout the supply chain. The presence and spread of *Salmonella* is complex and depends on numerous variables. As such, there is no single effective control measure. Further, as no one business through-chain operates in exactly the same way, the identification, level and management of risks will vary. This means that there is no one 'best practice', and that each business must identify 'appropriate practice' for managing identified *Salmonella* risks. These issues make the control of *Salmonella* a complex issue subject to a combination of both real and perceived risks.

There is an abundance of peer-reviewed and grey-literature (related to IP) regarding many aspects of *Salmonella*, however, the type and availability of this information varies through-chain. Therefore, there is an obvious need to collate readily available information and make it more accessible to all stakeholders through-chain, to provide a framework for the application of knowledge.

Currently, the level of understanding of the issue of *Salmonella* and eggs varies widely across industry, government regulators/health officials, the food service sector, and the consumer community. As elimination of *Salmonella* from egg farms is impossible, the risk of human illness needs to be appropriately managed. However, as this is a technically complex issue with varying risks (and varying understandings of the risks) between on-farm and in-food service, an effective management strategy will need to: a) be a collaborative effort that relies on the development of good relationships between industry and government agencies / regulators; b) be founded on robust information; c) involve a system (or systems) to control highest risk *Salmonella* spp. at various stages in the supply chain.

Salmonella is an organism that is ubiquitous in the environment as well as in a number of hosts (the type of *Salmonella* will depend on which host is affected). To date, there have been over 2500 identified types of *Salmonella* identified. Not all of these can be found on poultry farms and not all types of *Salmonella* found on poultry farms are pathogenic to humans (*104*). It is plausible to conclude that greater scrutiny of any stage through-chain would result in a greater level of *Salmonella* detection given its ubiquitous nature. However, there are a few risks that can substantially increase the risk of humans becoming ill from foods made with egg products. Fortunately there are also simple interventions available to address these risks.

All stakeholders have a responsibility to identify and address their *Salmonella* risks, however, this in itself would be an insurmountable task without having access to appropriate information. Common risks that are present at each stage through-chain, and that highly impact on *Salmonella* presence and growth include:

- grossly dirty and cracked eggs entering the grading area
- presence of yolk
- temperature above 7 degrees C
- presence of insects, rodents and animals
- presence of moisture on eggs/eggs coming into contact with wet surfaces
- compounding mechanical stressors that crack eggs
- cross-contamination of eggshells with other surfaces (including hands).

The safest eggs will be those that are removed from the hens and the environment as quickly as possible, and with at least these risks appropriately managed. Successful risk identification and assessment is about determining the most appropriate and cost-effective

strategy to manage a risk at each stage through-chain. An example may be identifying cleaning chemicals and/or facilities that may be able to be used in multiple areas. Risk management becomes expensive when procedures are continually added to existing procedures, without considering how risk management processes could be combined or streamlined.

It is also important to consider return on investment opportunities for all through-chain stakeholders to compensate for the additional costs that may be involved in improving *Salmonella* risk management. This is similar to the 'Lion Egg' initiative undertaken by the UK egg industry, which developed a market for eggs that were produced to the highest standards of *Salmonella* risk management. This program shifted industry and consumer attitudes to eggs and food safety. As a result, the Lion Egg scheme reports that approximately 90% of egg production in the UK is under this program.

There is little doubt that the contamination of poultry products (both meat and eggs) is a high contributing factor to human salmonellosis cases, and that *Salmonella* species can be found in a variety of food groups (*81*). However, it has proven difficult to measure public health outcomes of changes to food hygiene regulation (*217, 218*).

There are numerous types of *Salmonella* present on egg farms in Australia, with the most important human pathogenic type being *Salmonella* Typhimurium (*40*). In the USA a risk assessment has been performed on the presence of *Salmonella* Enteritidis (*96)* and the World Health Organisation published its risk assessment for *Salmonella* in eggs in 2002, which also predominately focussed on *S.* Enteritidis (*240*).

A risk assessment performed by researchers at the University of Adelaide for the Australian egg industry in 2006 (*222*) recommended that:

a) introduction of new mitigation strategies into commercial flocks producing graded eggs was unlikely to reduce the risk of human salmonellosis;
b) food standards should be reviewed to minimise the use of cracked and ungraded egg products in food for immune-compromised individuals;
c) refrigeration of eggs after processing and during wholesale and retail storage could substantially reduce the risk of human salmonellosis (this was found to have had the greatest impact on the risk reduction model used compared to other strategies); and
d) general improvement to hygiene and food storage practices in catering operations is required to prevent cross-contamination and temperature abuse of egg products.

This report is attached as an appendix. After consideration of the risks identified, and the research available during the development of the *Salmonella* Initiative report, these recommendations are supported.

Other livestock industries have invested considerable funds in identifying risks and performing hazard-based risk assessments (*43, 97, 98*), and a review of published data found significant reduction in the presence of *Salmonella* in pork and chicken meat, but not beef, after the introduction of this type of food safety program (*242*). However, the egg industry is diverse in its operations and it may not be practicable to perform a hazard analysis beyond identification of the highest risk variables. Focus on minimisation of *Salmonella* in general at each stage through-chain is recommended as the control of a single type of *Salmonella* is suspected to result in the increased presence of other types of *Salmonella* (*69, 96*).

Since 2011, Australian State jurisdictions have reported significantly rising rates of human salmonellosis linked to the consumption of egg products in particular, which is reflected in the Australian Government Department of Health OzFoodNet reports (http://www.ozfoodnet.gov.au/). Specifically, OzFoodNet estimated that in 2011 approximately 20% of foodborne illness outbreaks that were investigated were linked to the use of raw or low-cooked egg products. In 2010, OzFoodNet reported 30,035 notifications of nine diseases or conditions that are commonly transmitted by food, with the most frequent being *Campylobacter* (16,968 notifications) and *Salmonella* (11,992 notifications). The majority of notifications constitute 'sporadic' cases where no obvious cluster of cases is found, which makes them incredibly difficult to investigate. Therefore, Department of Health resources are focussed on outbreaks. *Salmonella* Typhimurium was the most frequent type of *Salmonella* detected in Australian salmonellosis outbreaks (44%) (*2*).

The majority of foodborne gastroenteritis outbreaks (81%) were due to person-to-person spread, 9% were suspected or confirmed to have been transmitted by contaminated food, 9% had an unknown mode of transmission and one outbreak was due to transmission from animal to person. The most recent estimate of the total cost of all foodborne illness to the Australian economy is $1.2 billion per year (*86*), which includes the cost to the healthcare system and lost business productivity.

Foodborne salmonellosis was estimated to have increased by 24% between 2000 and 2010 (*122*). In 2010, *Salmonella* was the most common aetiological agent identified in foodborne outbreaks, and restaurants were the most frequently reported food preparation setting. A single food source was identified for 43 outbreaks, 21 of which were associated with the consumption of dishes containing raw or minimally cooked eggs, the majority due to *S.* Typhimurium (*2*).

There has been recent evidence to suggest that climate variables might also impact on the rate of human salmonellosis (*105*), however, there are numerous other factors that are involved. The rate of human salmonellosis linked to the consumption of poultry products has increased with population and consumption trend increases, however, there do appear to be a few compounding factors that have occurred during the past 5-10 years that may have contributed to this sudden significant increase:

- changes in diagnostics (*122*)
- higher notification rate (outbreak multiplier value changed from 15 to 7) (*122*)
- changes in consumer eating habits (eating out more, more catering, more organic/raw) (*118*)
- more 'fancy foods' / celebrity chefs (*22*)
- more cage-free egg consumption.

However, there are a number of issues with each of these possible causes. Specifically, initial assessment undertaken by AECL and OzFoodNet found that the way the human salmonellosis data and egg consumption data are collected meant that these data cannot be used to establish if the increase in demand for cage-free eggs is contributing to the increase in human salmonellosis cases.

Current egg consumption data only reflect the retail sales of eggs and are not indicative of the majority of salmonellosis cases that are linked to bulk food preparation through caterers or restaurants, which primarily purchase bulk packs of eggs. The assumption would be that the trend in retail consumer sales of eggs of a particular farming type would be a trend reflected in food service/catering. However, consumers are not able to choose which eggs they consume at a food service/catering level nor can they often choose the level of heat application to the final egg product, which can destroy *Salmonella*) as they can in the home setting.

There are published articles that conclude that either free range or cage eggs are a higher risk for *Salmonella* (reviewed in (*237*)). Free range can be higher risk due to the ability of the eggs to come into contact with mud/dirt/faeces and because they can spend much longer in close contact with the hens (*109*). Cage eggs can be higher risk as flock density is a key variable for higher *Salmonella* risk. High density would also apply to some types of free range production where the hens are confined at night in housing, as the free range flock density refers to outside space available to the hens, not inside space. Therefore, there needs to be movement away from discussion about whether free range eggs or cage eggs pose a higher risk for human salmonellosis, as the ultimate risk factor will be the quality of the flock management. There is no argument that cage-free farming systems are more difficult to manage than cage systems, however, cage eggs still supply the bulk of the retail market and, in particular, catering and food service outlets are more likely to buy eggs based on cost, than on farming system (unless they want to promote this fact), and foodborne illness outbreaks are more likely in catering or food service venues.

A USA investigation into the rate of egg contamination at the farm level found that there was a significant difference in the rate of egg contamination between serovars, with *S.* Enteritidis causing a higher rate of contamination of egg contents and a lower rate of contamination of eggshells compared to non *S*. Enteritidis (including Typhimurium) serovars (*4*). There appears to be a linear relationship between the rate of contamination of egg contents and the percentage of a flock infected with *Salmonella*, but a nonlinear relationship between infection prevalence and the rate of eggshell contamination, with eggshell contamination occurring at a much higher rate than that of egg contents.

Eggshell contamination can occur in a number of different ways (e.g. the eggshell can come into contact with contaminated surface or dust can settle on the eggs) so it would be difficult to assess the exact risk of eggshell contamination alone as a function of percentage flock infection (*4*). A sample of 18 flocks predicted a contamination rate of 0.24% for internal contents and 0.53% for eggshells from eggs collected on farm. This prediction would equate to approximately one egg in every 10,600 harbouring *Salmonella* on the eggshell. However, this study was performed in the USA and there are some key differences between the USA and Australian egg industries that could impact on the appropriateness of extrapolating these results to the Australian situation.

A South Australian Research and Development Institute study of retail eggs conducted in Australia in the early 2000s found no *Salmonella* on or in 20,000 eggs (*222*), however, a retail survey of various foods including eggs in South Australia found *Salmonella* on the eggshell of 3.5% of the egg samples tested (one sample constituted a dozen pack). Another Australian study concluded that the occurrence of *Salmonella* on eggs in Australia was relatively low (*37*). International studies have estimated eggshell contamination at 0.05-0.19% (*121*), 0.18-0.4% (*47*) and 1.05% (*32*).

The contamination rate will depend on numerous variables including flock management and *Salmonella* serovar (*104*). There is often analysis of *S.* Typhimurium auxiliary to *S.* Enteritidis investigation due to its common presence on egg farms globally. However, there remain considerable knowledge gaps regarding how *S.* Typhimurium and *S.* Enteritidis differ, as the bulk of the research investigates *S.* Enteritidis as a priority given its ability to infect the internal contents more easily than *S.* Typhimurium (*235*).

Appropriate management of *Salmonella* risk can only be achieved through a quality understanding of:

- *Salmonella* in general
- what constitutes a risk (how to identify hazards)
- what constitutes appropriate management
- where to access accurate information
- QA processes and how to demonstrate/verify that the process meets QA requirements (Guideline for the Validation of Food Safety Control Measures; Codex CAC/GL-2008)
- risk variation (e.g. season to season, flock to flock).

However, this is an extensive list of required understanding for any through-chain stakeholder. The AECL *Salmonella* Initiative has engaged with numerous key stakeholders, including state regulators/health departments, industry associations, egg producers, researchers and technical consultants (including veterinarians), to identify and collate information regarding *Salmonella* risk management, so that appropriate interventions are able to be implemented through-chain without large impost on any business, yet providing a high level of intervention.

Given the extensive number of stakeholders and the fluid nature of *Salmonella* information this consultation process, and by extension this document, should not remain static. Rather, it should be regularly updated to ensure that any new information that may impact on *Salmonella* risk management anywhere through-chain can be better managed. It is imperative that all stakeholders are able to comment on what might be considered appropriate minimum management, while providing supporting data and/or peer-reviewed work. The minimum considerations provided here are designed to stimulate discussion among relevant stakeholders as to what the accepted minimum considerations should be. The minimum considerations listed in the tables in this report have been developed with relevant stakeholder input, however, they should not be considered the final version as further consultation with key industry stakeholders is required.

Food safety is a process of continual improvement. This report should be used to initiate discussion between through-chain stakeholders to determine what constitutes appropriate management of *Salmonella* at each stage through-chain. The ultimate outcome is a user-friendly online decision-tree based tool that provides all stakeholders with a starting list of risks to consider for their particular operation. The aim of the 'why it is a risk, how to control it and other considerations' column is to improve the understanding of each stakeholder as to what constitutes a *Salmonella* risk and how to identify all risks at each stage beyond what is mentioned here.

The aim of the first 12 months of the *Salmonella* Initiative was to coordinate, review, identify and disseminate comprehensive yet coherent information regarding *Salmonella* and *Salmonella* risk identification and management through-chain. It is not feasible to expect that a raw animal product that comes out of the hen through the same opening as the faecal matter could ever be sterile, so the risk of *Salmonella* will always be present. Therefore, the ultimate aim is to reduce *Salmonella* load through-chain.

NB:

1. State jurisdictions interpret and implement the FSANZ Standards, and may do so differently. State legislation will reflect this and must be taken into consideration. FSANZ Standards referred to in the tables below are the reworked version that comes into effect in March 2016, however, there have been no notable changes to

the standards relating to egg production (2.2.2 and 4.2.5). The new version of the Standards have been included to ensure the references are as up to date as possible at the time of publication of this report.

2. The references in the tables below do not necessarily represent the entire cache of available information for each risk, as is in some cases there was a considerable amount of research available and only the most relevant were selected for inclusion.

2 Through-Chain *Salmonella* Risk Identification

2.1 On-Farm

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Farm management	Cage / Barn / Free range	The farm / flock management must be of a high standard as this determines the level of *Salmonella* in a flock and/or on eggs, not the production system Producers must understand FSANZ standard 4.2.5 and 2.2.2	• Cage / Barn – high risk due to high flock density, especially if some birds have already been infected with *Salmonella*. Caged layer house is harder to clean and disinfect effectively compared to other farming systems. • Free range – *Salmonella* risk due to prolonged interaction between the egg and the environment after the hen has laid the egg. **Note:** Producers need to understand: • FSANZ Standard 2.2.2 – Eggs and Egg products • FSANZ Standard 4.2.5 – Primary Production and Processing Standard for Eggs and Egg Product (especially clause 9, which states "*An egg producer must not obtain eggs for human consumption from birds if the proprietor, supervisor or employee of the egg producers **knows**, **ought reasonably to know** or to **reasonably suspect** the bird is affected by disease or a condition that makes the eggs unsafe or unsuitable.*").	112, 170, 224, 237, 241 FSANZ 2.2.2 FSANZ 4.2.5 Recommendation: Review FSANZ standard 4.2.5 and consider removal of clause 9 or provide more clarity on producer responsibility.

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Receiving chicks / pullets	Cage / Barn / Free range	Chicks / pullets should be received from a source that has adequate *Salmonella* risk management in place	• Chicks / pullets / hens that are received from suppliers should have adequate *Salmonella* risk management in place to reduce the incidence at the laying site and therefore reduce *Salmonella* infection and shedding during lay (and by extension, *Salmonella* contamination on the eggs). • Suppliers should have a certificate or statement of the *Salmonella* controls undertaken by the business supplying the chicks or pullets or results of any *Salmonella* testing on the flock. • *Salmonella* infection and shedding may be able to be controlled through vaccination. However, it may involve a number of vaccination administrations. • If pullets are placed on a farm it is important to understand whether additional vaccinations are needed to complete the required vaccination schedule, to ensure maximum vaccine efficacy. • Different types of *Salmonella* vaccines are available overseas for poultry administration, however, only one is produced domestically. • Varying efficacy of international *Salmonella* vaccines is noted. Vaccination may not significantly impact on the level of bacterial shedding during hen stress.	6, 7, 39, 57, 84, 88, 137, 150, 163, 229 AECL 1US091 *Salmonella* control in layer chickens

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Vehicle and equipment movement onto farm	Cage / Barn / Free range	Limit movement of vehicles, equipment and machinery onto the farm Assess whether dead bird retrieval, waste removal, feed delivery and/or other deliveries can be limited to outside the perimeter fence	• Have designated vehicles and equipment • If unable to have designated vehicles / equipment, implement quarantine interventions including vehicle movement only one way in one day, install a designated vehicle sanitising station, and ensure that machinery, equipment or vehicles entering the farm are clean. • Ask the vehicle owner (if not owned by the producer) for decontamination SOPs that are used. • Vehicle movement is not always able to be directly controlled, so consideration should be given to controlling access areas for these vehicles and equipment to limit environmental contamination. • If there are multiple farm locations in a single business, limit cross-contamination between flocks.	42, 134, 135, 169, 187, 208 AECL Biosecurity Code of Practice Animal Health Australia National Farm Biosecurity Technical Manual for Egg Production
Pest control – insects and rodents	Cage / Barn / Free range	Minimise insect load near hens / hen housing Adequately and regularly monitored pest control program	• As far as possible, minimise insects (including flies, beetles and cockroaches) and rodents (including pet rodents) from inside the housing. This will control hen *Salmonella* infection that can occur either directly through contact or indirectly through feed and water contamination. • There is a correlation between *Salmonella* load in a hen population and the density of the rodent population, therefore rodents must be controlled. • Rodent bait stations must be monitored and re-baited regularly and hens must not have access to the baits, so that they can't ingest the rodenticide.	58, 70, 80, 94, 104, 123, 127, 145, 185, 188, 219, 225 AECL Final Report DAQ-244J *Investigations into the management of the darkling beetle*

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Animals – domestic and wild	Cage / Barn / Free range	Secure hen housing from unwanted access to animals	• Animals - including chickens, turkeys, dogs, cats, pigs, cows, sheep, wild birds (including ducks, pigeons, finches and sparrows) and other wild animals (e.g. foxes) - are carriers for various types of *Salmonella*. • Hens can become infected with *Salmonella* if their environment is contaminated by infected animals. This risk is heightened when they come into contact with and/or ingest, faecal material from infected animals. • Reduce the attractiveness of water reservoirs to water fowl (e.g. ducks) - such as having no attractive irrigated grass, no dams without netting, and an active duck repelling program. • If animals such as dogs or alpacas are used to protect the hens, they should be tested for *Salmonella* prior to introduction to the flock and these animals should be retested regularly.	20, 28, 83, 103, 104, 121, 151, 165, 174, 203, 231
Perimeter fence, fencing	Cage / Barn / Free range	Perimeter fence must be present either around the entire farm operation and/or around different farm systems	• Fencing will limit unwanted entry on to the farm by land animals and people that can carry *Salmonella* and infect environment/flocks. • Fencing can prevent free range hens and debris coming into contact with a cage/barn system. • The main entrance for the perimeter fencing should display appropriate signage and be lockable.	169 AECL Biosecurity Code of Practice Animal Health Australia National Farm Biosecurity Technical Manual for Egg Production

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Farm staff training	Cage / Barn / Free range	All staff must be trained in food safety, with at least one regular staff responsible as a food safety manager	• Staff must be trained in food safety to reduce the risk of human salmonellosis linked to eggs that they handle, through proper handling / storage / transport, and made aware of its importance to the reputation and profitability of the business should be stressed. • Encourage staff to assess their tasks to identify areas that could be continually improved. • Humans can carry pathogenic *Salmonella* in their gut and may be able to contaminate hens. This is particularly important for staff returning from international travel. • Staff with an intestinal upset, must not have contact with hens or eggs for at least 48 hours after cessation of symptoms (about 50% of adults infected with *Salmonella* will continue to excrete for over 5 weeks and 10% will excrete for over 9 weeks). • Maintain a regular toilet cleaning schedule and ensure that hand washing and hand sanitising facilities are available throughout the production area.	101, 120, 121, 147, 172 FSANZ 4.2.5

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Farm staff and visitor biosecurity, including domestic birds	Cage / Barn / Free range	Farm staff / visitors must not have access if they have been to another farm or been in contact with hens from outside of the farm Farm staff / visitors must not have handled avian pathogens within the previous 48hr period Farm staff should be prohibited from owning domestic birds at their place of residence Visitors must declare if they own birds before coming onto the farm	• *Salmonella* can be transferred from domestic birds, farm environments and laboratories to humans and their environment. • If farm staff / visitors become infected in this manner then the infection could potentially be transferred to hens or eggs. • This is very important for birds that have outside access. • Domestic animals such as cats, dogs, etc., are known to carry *Salmonella*, hence these animals can also transmit Salmonella to humans and potentially to hens and eggs, and should be considered as a risk.	147, 169 AECL Biosecurity Code of Practice Animal Health Australia National Farm Biosecurity Technical Manual for Egg Production

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Human traffic	Cage / Barn / Free range	Limit human foot traffic on the farm and through areas where hens are present (except regular farm staff) Have dedicated farm / shed boots, or boot dips Have dedicated farm clothing or over-suits and hairnets	• *Salmonella* can be present on clothing, hair, skin and footwear, therefore limit contact of the hens with these vectors. • Have dedicated farm / shed boots, boot covers (not advisable as they are damaged easily) or boot dips, dedicated farm clothing or over-suits and hairnets. • Regular farm staff should often walk through all areas where the hens are present so the hens can become acclimatised to human movement and are not stressed by this activity (stress significantly increases the risk that the hens will shed high levels of *Salmonella*).	169 AECL Biosecurity Code of Practice Animal Health Australia National Farm Biosecurity Technical Manual for Egg Production USA Food and Drug Administration 2011 *Prevention of Salmonella Enteritidis in shell eggs during production, storage and transportation*
Boot dips / foot baths (dry and wet)	Cage / Barn / Free range	Assess chemical content regularly and change as required	• Ensure that the concentration for all boot dips / foot baths is correct, and check concentration with test strips. • The location of the boot dip and the chemical used are important, and it may be pertinent to have a pre-dip to remove mud and excess dirt from disrupting the chemical efficacy. • Rain will dilute the chemical and therefore the efficacy. Hence, need to assess more regularly, or cover the boot dip / bath. • The quality / hardness of the water can impact on the efficacy of the chemical. • Dry foot baths utilizing dry and/or aerosol disinfectants may be	52, 117, 133, 154, 169, 179, 190, 216 AECL Biosecurity Code of Practice Animal Health Australia National Farm Biosecurity Technical Manual for Egg Production

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			used in lieu of boot dipping stations. • Consider farm and/or flock specific boots for visitors and staff. • Check efficacy of footwear treatment (dirt removed / soaked with active chemical in boot treads).	USA Food and Drug Administration 2011 *Prevention of Salmonella Enteritidis in shell eggs during production, storage and transportation*
Debris and vegetation around the housing	Cage / Barn / Free range	Maintain vegetation regularly to limit coverage for insects and rodents	• Remove any cover for rodents near the housing to reduce the organic load, insects and availability of nutrients for bacterial growth (particularly if water is able to pool). • Control of rodents and organic load around the shed will reduce *Salmonella* and in free-range systems, this will also improve management of dust movement through the housing and reduce rodent entry into the hen housing. • Keep grass and vegetation short for an appropriate radius around the permanent housing (anecdotally a 1.5m radius has been effective, and the immediate radius can be replaced with rocks). This not only removes cover for rodents and wild animals, but also reduces the incidence of undetected floor eggs in free-range systems. • The vegetation limited / free radius around the hen housing will need to be assessed for what is appropriate in each operation.	70, 169 AECL Biosecurity Code of Practice Animal Health Australia National Farm Biosecurity Technical Manual for Egg Production

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Hen housing orientation and layout	Cage / Barn / Free range	The orientation and distance between buildings should be appropriate to minimise pathogen spread and transmission	• *Salmonella* is present in dust and must be controlled. • Dust, including hen organic matter and other particulates, can move via air movement and ventilation (air being drawn into other hen housings). • Considerations should be given to the distance and barriers, etc. between hen housings to limit spread of *Salmonella* between flocks in different housings. • Hen housing orientation alone will not eliminate the risk of *Salmonella* spreading / contamination, although measures can be put in place to reduce the risk(s).	35, 78, 169 AECL Biosecurity Code of Practice Animal Health Australia National Farm Biosecurity Technical Manual for Egg Production USA Food and Drug Administration 2011 *Prevention of Salmonella Enteritidis in shell eggs during production, storage and transportation*
Hen housing and equipment (incl. tiers, fans, belts, etc.) – all in / all out	Cage / Barn / Free range	Cleaned and sanitised effectively and verifiably after hens have been removed	• Hen housing should first be dry cleaned of organic materials, followed by a wet wash with a free-rinsing foaming agent, high pressure wash to remove the soils, then rinse. A sanitiser should then be applied (preferably one that is active in the presence of organic matter). Good housing drainage is also required. • The type of chemicals used will depend on what needs to be cleaned (different chemicals have different actions and the chemicals must be compatible with each other if used on the same surface, and must be compatible with the material of the shed / equipment (e.g. concrete or aluminium).	34, 35, 49, 50, 52, 78, 117, 133, 148, 154, 173, 190, 216

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			• A good chemical can be simply applied, and will penetrate all the components that can be present from an egg production, and then rinsed off. • The chemical(s) used must be able to solubilise / emulsify fats (requires sequestrants / emulsifier), penetrate soils / dirt / faeces (surfactant), breakdown proteins (e.g. yolk and albumin), and maintain all of these components in solution so that it can be rinsed off easily and not reattach to the shed or equipment. • Cleaning needs to be done quickly or in sections, to avoid chemicals and extraneous matter from drying up. • Build-up particularly occurs in building and equipment joins (e.g. where the wall joins the floor or where one component meets another), and can be difficult to remove. • The sanitiser needs to be compatible with all chemicals pre- and post-cleaning to ensure the efficacy of the sanitiser.	

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Hen housing and equipment (incl. tiers, fans, belts, etc.) – <u>multi-age</u>	Cage / Barn	Application of a multipurpose sanitiser that penetrates organic matter and reduces bacterial load – can be applied to a tier that has been depopulated Dry clean and/or wet clean other equipment and shedding regularly Good housing drainage required	• Cleaning the housing and internal equipment when hens remain in a section of the housing is difficult, but it is possible to reduce *Salmonella* (and total bacterial) load by dry cleaning and removing dust, dander and other extraneous materials such as faeces, on a regular basis. • If possible, perform sections of wet cleaning after flock depopulation. • The types of chemical used should be fit for purpose and be compatible with each other if used on the same surface (e.g. concrete or aluminium) / shed / equipment. • The chemical(s) used should be simply applied to penetrate all the components that can be present from an egg production, and then rinsed off. • The chemical(s) used must be able to solubilise / emulsify fats (requires sequestrants / emulsifier), penetrate soils / dirt / faeces (surfactant), breakdown proteins (such as yolk and albumin) and maintain all of these components in solution so that it can be rinsed off easily and not reattach back to the shed or equipment. • Cleaning needs to be done quickly or in sections to avoid chemicals and extraneous matter from drying up. • Build-up particularly occurs in building and equipment joins (e.g. where the wall joins the floor or where one component meets another), and can be difficult to remove. • There are some sanitisers that are approved for misting in the presence of animals, and may be able to be used as a method of partially sanitising a multi-age shed. • Alternatively a sanitiser can be applied to a whole vacant tier as a foam or low pressure spray without contacting birds in other	34, 35, 49, 50, 52, 61, 78, 117, 133, 148, 154, 173, 190, 216

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			tiers, provided that it is fit for purpose.	
Housing floors	Cage / Barn / Free range	The house flooring should be: an impervious material (such as concrete); a material that is easily cleaned thoroughly; or a surface that is raised above the impervious surface, constructed of a material that allows organic matter and dust to fall through (e.g. plastic slats) Any flooring should be cleaned regularly	• Flooring material should be cleaned, with adequate drainage to ensure that the cleaning solution and water are not able to pool and the flooring can dry adequately. • Drying reduces dust and other organic matter that can harbour *Salmonella*. • Anecdotally, in cage-free farming systems, raised slatted surfaces reduce the organic matter and dust in contact with the hens as this matter is able to fall through the slats onto the floor, and can be easily removed without disturbing the hens (if access is possible).	49, 50, 169 AECL Biosecurity Code of Practice Animal Health Australia National Farm Biosecurity Technical Manual for Egg Production

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Housing ventilation	Cage / Barn / Free range	Must be adequate to maintain airflow to reduce excess moisture Hen housing should be aligned to prevent cross-shed contamination via forced ventilation systems	• Faeces, especially wet faeces, are reservoirs for *Salmonella*. • Adequate airflow can help dry faeces as *Salmonella* does not grow well in a dry environment. • In cage-free systems, adequate ventilation can limit hens spreading wet faeces through movement and limits the incidence of floor eggs coming into contact with wet faeces. • Internal egg contamination can occur if eggs come into contact with wet faeces, particularly straight after lay because the internal temperature of the egg is higher than the temperature of the faeces, which will result in bacteria being drawn into the egg by convection. Moreover the cuticle, which can protect the shell from entry of bacteria, takes approximately 3 minutes to harden. • Water facilitates the growth of bacteria and the movement of bacteria through the eggshell, therefore wet faeces are high risk for internal egg contamination. • The direction of ventilation is particularly important for risk management in multi-age sheds, as tunnel ventilation draws air from one end of the shed to flow through the shed to cool hens. • Where the air source is drawn from, it should be minimally contaminated by other hens / shed outputs, and shed outputs into other sheds should be minimal. • Cross-flow forced fan ventilation is also a problem, so farms should be designed so that sheds next to each other exhaust on the same side and intake on the same side (not drawing exhaust straight into intakes).	35, 78, 104, 169, 209, 215 http://www.ncbi.nlm.nih.gov/pubmed/25791009 AECL Biosecurity Code of Practice Animal Health Australia National Farm Biosecurity Technical Manual for Egg Production USA Food and Drug Administration 2011 *Prevention of Salmonella Enteritidis in shell eggs during production, storage and transportation*

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Shed drainage and drainage around the shed	Cage / Barn / Free range	Hen housing should have appropriate drainage	• Drainage around the hen housing should be adequate to ensure that bodies of water do not form due to rain, water used during shed cleanout or water overflow. • Bodies of water may attract wild animals (including birds), which can transmit *Salmonella*. • Bacteria will proliferate where water / mud is present and in free range systems. If hens lay floor eggs onto mud, there is a risk of drawing bacteria into the egg. • Hens can transfer mud into the housing and contaminate the feed / water and nesting areas.	104
Pre-hen placement housing cleanliness	Cage / Barn / Free range	Assessment of cleaning efficacy	• A new flock introduced into a multi-age shed can stress existing birds. If the birds are already infected with *Salmonella,* this can result in *Salmonella* shedding and can transmit to a new flock. • It may be appropriate to test existing flocks in a multi-age shed for *Salmonella* prior to placement of a new flock. • For an all-in / all-out system, ensure that the shed is cleaned appropriately prior to hen placement. • Hen housing should be tested for the absence of *Salmonella* (along with other pathogens of importance), however, the timing of flock placement may not allow for results to be returned before the flock is placed. • If testing results are not back, a thorough visual assessment alone for freedom from organic matter should be the minimum requirement, as any bacteria present in the organic matter can infect new hens placed in the housing. • It is not expected that in commercial operations (besides breeder operations) the housing could be sterilised between	52, 117, 133, 154, 190, 216, 234

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			flocks, but efforts need to be made to reduce the bacterial load in the housing. • Quality of the water may limit the efficacy of the chemical(s) used to clean and sanitise. • Fresh disinfectants must always be used and organic matter must be removed before disinfection.	
Hen stress	Cage / Barn / Free range	Identify times of stress (such as hen transport and onset of lay) and have procedures in place to minimise hen stress, and deal with situations where stress is unavoidable	• Birds will shed *Salmonella* when they are stressed, as their immune system is supressed and this can increase percentage flock infection. Stresses can be due to: - onset of lay - transport / change to routine - pre-existing disease - moulting - high traffic areas - environmental conditions (e.g. heat, rain, high humidity, mud) - feeder / drinker breakdown (or any pause in feed and/or water access), etc. • Hens infected with *Salmonella* can shed the bacteria for more than a month after initial infection, which can lead to persistence of *Salmonella* in a flock. • *Salmonella* can persist in the gut without active shedding, with onset of shedding during a stress event, although diseases, stress and *Salmonella* shedding may be managed through a vaccination schedule. • Stress can limit cuticle deposition – a natural egg barrier to bacterial penetration through the shell. • In free range systems with moveable housing, the hens may	1, 19, 38, 65, 75, 92, 93, 95, 100, 110, 124, 136, 162, 178, 237

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			need to be moved regularly (e.g. once per week) to acclimatise them to this activity, which will reduce the incidence of stressful events throughout their laying period. • Differences in *Salmonella* infection rate and shedding have also been attributed to hen age and breed.	
Perches	Free range / Barn	If perches are present, they should be positioned in a manner that reduces the incidence of the hens defecating on each-other or into feed/water and nests	• Perches should be situated with enough distance for a mature size hen to face either way and not be able defecate directly onto a hen perched below or above (approximately 30cm). • Hens must not be able to defecate into feed and water, as this will contaminate key hen inputs and provide an environment for *Salmonella* and other bacteria to grow, especially if the faeces are moist / wet. • Nests need to be designed so that birds do not perch on the edges and defecate into the nest. • *Salmonella* can persist in feed. • There is some indication that *Salmonella* has specific genes that allow the pathogen to survive in the acidic environment of faeces.	23, 47, 49, 89, 104, 170
Compounding mechanical stress	Cage / Barn / Free range	Assess entire egg process for mechanical stresses to the egg	• Mechanical stress can occur when an eggshell comes into contact with another surface (e.g. moving contact with other eggs or equipment, or onto the egg belt) and can cause fractures in the eggshell. • Fractures can vary from those that are undetectable by automated detectors to those visible-by-eye. • Visible cracks or fractures are considered high risk for enabling	5, 27, 36 184, 227, 228 FSANZ 2.2.2

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			easy access for bacteria (e.g. *Salmonella*) to enter the egg, as well as being a farm profitability issue. **Note: "*visible, or visible by candling or other equivalent methods*" are prohibited from sale under FSANZ Standard 2.2.2.** • Egg processes should determine if there are sections of the process that can reduce the incidence of mechanical stress. • Studies have shown that cracked eggs alone are not of greater risk for spoilage than normal eggs. • Unwashed cracked eggs and cracked eggs washed in clean water were apparently as resistant to spoilage as uncracked eggs receiving the same treatments. • Inappropriate washing processes substantially increased spoilage of cracked eggs compared to normal eggs. • This type of research would need to be repeated in the current commercial environment. • No comprehensive study could be found that investigated what potential quantitative increase in risk of *Salmonella* contamination cracked eggs pose. • This could be because it is difficult to define a cracked egg and *Salmonella* can easily transfer through intact shells. • A number of industry risk assessments have concluded that cracked eggs pose a high risk of human salmonellosis if consumed. • The level of pressure required to crack an egg (hairline crack or greater) will vary depending on the quality of the eggshell, which can be affected by many variables such as hen health,	

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			age, feed formulation and breed.	
Manure belts	Cage / Barn / Free range	The belts should be emptied at least twice weekly Ensure that scrapers are functioning every time the manure belt is moved, and clean appropriately	• Empty the manure belts often to prevent build-up and potential contact of faeces with the birds and eggs. Manure belts should be cleaned regularly. • In cage systems, if the hens in the cage below can reach and peck the manure belt from the cage above, they can ingest faecal matter that contains *Salmonella*. • Effective scrapers are required to remove / reduce faecal build-up on the manure belt, to reduce organic load and therefore bacterial load in the housing. • Replacement motors for the belts should be available to ensure fast turnaround in the event of breakdown.	38, 112
Manure dryer (if applicable)	Cage / Barn / Free range	The manure dryer must be maintained appropriately	• If a manure dryer is available, use it to keep the moisture level in the manure to a minimum. • Low moisture level can limit (and potentially inhibit) bacterial growth, which will reduce the overall bacterial load in the hen housing. • Currently, there is no specific research showing whether this type of intervention will be effective, although generally *Salmonella* will grow rapidly if moisture is present.	38, 104

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Presence of yolk	Cage / Barn / Free range	Reduce the presence of yolk, particularly on equipment that has regular / sustained contact with eggs (e.g. egg belts, nest pads) Clean and sanitise	• Egg yolk is an excellent growth medium for *Salmonella.* • Egg yolk must not be present on equipment that is in regular contact with eggs, such as egg belts or nest pads, to limit direct contamination of the eggs. • Antibodies present in the yolk have been shown to have little effect on the growth of some types of *Salmonella* but other studies have indicated there may be some inhibitory effect. • The efficacy of antibodies may be affected by a number of variables.	17, 31, 73, 74, 104, 141, 195, 220
Presence of dust / feathers / faeces / extraneous matter	Cage / Barn / Free range	Maintain a regular schedule to remove dust and other extraneous matter from equipment, floors and housing during lay	• A regular dry cleaning schedule to remove dust and other extraneous matter will prevent build-up and reduce hen infection, and reduce overall *Salmonella* load in the hen housing. • Dry cleaning could involve sweeping or vacuuming. • Hens can be trained early to be familiar with the noises and movement associated with this cleaning, to reduce incidences of stress, which can cause hens to shed high levels of *Salmonella*. • Compressed air can remove / reduce the dust and organic matter build-up, but it will increase the spread of bacteria through the hens as compressed air will aerosol the bacteria.	38, 49, 78, 134, 164, 173, 181, 234
Water / moisture on eggs	Cage / Barn / Free range	Eggs must be dry at any time (besides during a strictly controlled washing process)	• Eggs must be kept dry at all times (except during a controlled egg washing process), as water facilitates the movement of *Salmonella* into the egg and the rate of penetration is determined by the quality of the eggshell and cuticle. • Cooling systems should not allow eggs, or equipment that eggs	16, 53, 77, 104, 148, 189, 193, 241

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			come into contact with, to become wet or produce condensation (referred to as 'sweating'). • Diffusion into the egg can occur due to differences in density / pressure due to temperature differences between the internal egg and the outer environment. • Any external application to the egg must be at a temperature higher than the internal temperature of the egg (including the eggshell) so that diffusion is from inside the egg to outside. • If an egg is laid into a wet environment or comes into contact with water that is at a lower temperature to the internal contents (an egg is laid at approximately 41°C), the result is diffusion from the outside to the inside of the egg, i.e. transfer of *Salmonella* on the surface of the egg to the inside. • Eggs should be slowly cooled to the final storage temperature through the on-farm process and not subjected to fluctuations in temperature. After which, to limit condensation on the eggs, the cold-chain must be maintained once they have reached the required storage temperature. • Egg conveyors must be covered at all times, especially if part of the conveyor is outside, to ensure that rain cannot land on the eggs.	

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Staff handling dead or live hens, faeces or ungraded eggs	Cage / Barn / Free range	Hand-washing / sanitising facilities should be closely available for staff to use after handling live or dead hens, or faeces or pre-graded eggs, or coming in contact with dust	• *Salmonella* can be transferred onto hands from any contaminated live and dead hens, or faeces, dust or ungraded eggs. • Staff handling live or dead hens, faeces or ungraded eggs must wash and sanitise their hands after completion of the task, and especially before handling hens or eggs from another housing, to prevent cross-contamination and spreading of bacterial infection. • There is a high risk that ungraded and/or unwashed eggs will have *Salmonella* present on the eggshell surface. • The best hand hygiene technique is the combination of hand washing (to clean) and alcohol-based gel (sanitiser). • Improper / ineffective hand-washing can lead to a high risk of bacterial cross-contamination. • Antibacterial wipes and/or gel could be assessed as part of this procedure if applicable. • Flocks should be assessed daily for dead hens so they can be removed promptly, as they will attract insects and rodents which can transmit *Salmonella*. • Dead hens need to be disposed of in a manner that is not accessible to pests (particularly rodents).	8, 54, 78, 104, 106, 173
Raised floor with slats and litter on floor during production	Free range / Barn	The condition of the litter should be maintained in an acceptable quantity and of acceptable quality	• The litter needs maintenance to limit the proliferation of bacteria and reduce the bacterial load in the hen housing. • Where the flooring is part litter and part slats, there needs to be additional consideration as to how to reduce the incidence of hens transferring dirt / litter / mud into the nest boxes.	78, 104, 112, 173

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			• Eggs that are laid in the nest boxes should not come into contact with dirt / litter / mud, as these can carry *Salmonella.* • Any wet spots in the litter (caused by drinking water or wet faeces) should be removed regularly. • The litter produces dust, which aerosols when the hens are digging and moving through it, and this increases the risk of *Salmonella* spread. • Consideration should be given to how to distance the nest boxes from the designated area for the hens to lay their eggs (or whether this is appropriate). • Water will promote *Salmonella* growth, and the number of floor eggs should be minimised by training the hens to lay in nest boxes. There should be regular walk throughs to collect eggs laid on the floor, to disturb birds and discourage them from laying there.	
Eggs not laid in the dedicated nest boxes / floor eggs	Free range / Barn	Train hens to use nest boxes and to discourage them from laying elsewhere (i.e. 'floor eggs')	• Floor eggs have a higher risk of bacterial contamination because they come into contact with much more dirt / dust / faeces / moisture than eggs laid in controlled areas. • If the nest boxes are maintained regularly, and egg faecal contamination and contact time with the hen is limited, then the risk of bacterial contamination can be reduced. • Some breeds of hens may be at higher risk of laying eggs on the floor than other breeds. • Hens can be trained to lay eggs in the nest boxes by enticing them into the nests prior to initiation of lay, to provide them with adequate time to learn to use the nests, preventing their entry during non-lay periods (i.e. at night) and discouraging egg	14, 35, 38, 104, 112, 148, 173, 180, 189, 193, 205, 209, 215

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			laying in areas that are not optimal, such as outside in the dirt / mud / faeces. • Options include minimising outside stimulation in the pre-lay and initial lay periods, electric fencing, stimulation placed in the boxes prior to lay, and closing nest boxes at night. • Nest lights can be used to train birds to explore the nests but should not be left on during peak laying periods during the day. • Once the hens have learned how to use the nest boxes and floor egg issues are under control, then the design of the range, etc., can be amended. • Faeces are a reservoir for *Salmonella*. • Feed and water should be located within the hen housing to limit hens from laying eggs outside. • Diffusion into the egg can occur due to differences in density / pressure, because of temperature differences between the internal egg and the outer environment. • Any external application to the egg must be at a temperature higher than the internal temperature of the egg (including the eggshell), so that diffusion is from inside the egg to outside. • If an egg is laid into a wet environment or comes into contact with water that is a lower temperature compared to the internal contents (an egg is laid at approximately 41°C), the result is diffusion from the outside to the inside of the egg, i.e. transfer of anything on the surface of the egg (including bacteria like *Salmonella*) into the egg. • Research has found that it is easier to artificially contaminate (penetrate) eggs with a wet solution of faeces containing *Salmonella* than with a dry faecal sample.	

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			• The rate of bacterial penetration into the egg will depend on a number of variables including hen age, shell quality (which can be affected by age, health status and diet), hen breed and *Salmonella* type. • More research is required into the efficacy of specific interventions for reducing floor eggs. However, there are a number of variables that would affect any research-based understanding of efficacy (such as flock management, climate / weather changes). • Consider diverting floor eggs for pulping, to reduce the risk of contaminated floor eggs becoming part of table egg production.	
Extraneous material in nest box during production	Free range / Barn	Assess the nest boxes at least once every 24 hours and remove / reduce faeces, without spreading faeces to eggs during collection	• Nest boxes that come into contact with eggs should be free of extraneous matter, including faeces and egg yolk. • Remove faeces from nest boxes as regularly as possible without contaminating the eggs. • Eggs in contact with faeces for any length of time, or rolling over faeces, have an increased risk of *Salmonella* contamination; there is a higher risk of bacterial penetration into the eggs if water also present. • The amount of faecal contamination can be reduced if the nests are closed at night and the nests / egg conveyor are cleared before the hens' sleep, as this will prevent them from roosting in the nest boxes and leaving substantial quantities of extraneous matter in the nest boxes. • Assess whether the eggs are able to rollaway immediately or if modifications can be made to the nests to allow the eggs to rollaway. • Limiting faecal contamination on the eggs will reduce issues	104, 111, 112, 209, 215

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			during grading and, if applicable, washing. This may involve replacing highly dirtied nest box material. • Consider measures to train the hens to access the boxes for laying only. • Faeces are a reservoir for *Salmonella*, and yolk is an ideal growth medium for *Salmonella.*	
Nest box material not maintained during production	Free range / Barn	The nest box material should be clean before the flock is placed, should not be absorbent, and should be able to be easily cleaned and/or replaced as required.	• Nest box material that comes into contact with eggs should be free of extraneous matter including faeces and egg yolk. • How often the nest box material is replaced, cleaned or replenished depends on the efficacy of the material to reduce the incidence of eggs rolling through and lying on faeces and other extraneous material that can harbour *Salmonella*. • Materials can include sand, shell grit, straw, sawdust, wood shavings, carpet, plastic mats, fake turf, steel mesh. **Note: it is important that the material is not absorbent, to prevent bacteria embedding into the material and proliferating as this can contaminate the eggs with bacteria if they roll through it or otherwise contact it.** • Faeces are a reservoir for *Salmonella*, and yolk is an ideal growth medium for *Salmonella*. • Nest box material should be kept dry and not allowed to come into contact with any water source as the presence of moisture significantly increases *Salmonella* growth.	78, 104, 112, 141, 173, 195, 209

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Nest pads not cleaned / replaced regularly during production	Free range / Barn	Assess each day for build-up of faeces and cleaned / replaced on a regular basis	• Nest pads that come into contact with eggs should be free of extraneous matter including dust, faeces and egg yolk, which can all contain *Salmonella*. • If extraneous matter is present on the nest pads it can come into contact with eggs laid in the nests and any extended contact of the eggs with faeces increases the risk of bacterial transfer onto/into the eggs. • There should be a cleaning and sanitizing program in place for the pads after removal from shed, and spare pads handy to replace those removed for cleaning. • An appropriate cleaning schedule may be once per week to reduce build-up. • Nest pads must be kept dry at all time, as any moisture significantly increases *Salmonella* growth. • Faeces are a reservoir for *Salmonella* and yolk is an ideal growth medium for *Salmonella*.	78, 104, 112, 141, 173, 195, 209
Egg collection belts and conveyors	Cage / Barn / Free range	Cleaned / sanitised and dried on a regular basis	• Egg collection belts / conveyors that come into contact with eggs, should be free of extraneous matter including dust, faeces and egg yolk. • This can be difficult during the production day, but it is important to assess how build-up can be reduced. • If appropriate for the set-up, the collection belts can be dry cleaned of faeces and internal egg material during running of the belts by using a clean brush that doesn't damage the material. • Daily dry cleaning (including vacuuming) can be followed by sanitisation and drying to ensure the belts / conveyors are	78, 104, 112, 173, 214, 233

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			cleaned and dried before coming into contact with eggs. • At shed cleanout the belt should be wet washed and high pressure washed and sanitised, but it must be dry before eggs come into contact with it again to avoid eggs becoming wet. • The growth of *Salmonella* is significantly greater in the presence of water / moisture, and *Salmonella* can persist and be found in high levels in dust. • Spot cleaning can be performed using paper towels (or equivalent) to remove gross material, and a spray bottle (with cleaner / sanitiser). • All surfaces that come into direct contact with eggs should be kept clear of visible organic matter to reduce bacterial load on the equipment that can be transferred onto the eggs. • Assess whether the egg belts are able to be run prior to when the hens start laying eggs each day, so that the dust has a chance of being removed before eggs are laid. • Even if birds are not in production yet, the egg belts can still be run every day to minimise the build-up of dust and faeces (and acclimatise the birds). • Egg belts are an ideal place to test for the presence of *Salmonella*, as *Salmonella* can persist on the belt (the material used for the egg belt was the most important factor influencing *Salmonella* colonization and persistence). • *Salmonella* can also persist on the conveyor, and some materials used for conveyors are able to retain more bacteria than others (e.g. cloth and plastic). • Eggs must not get wet at any time except during a closely monitored washing process. Conveyor sections that are outside	

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			are open to rain and must be effectively covered. • The presence of water facilitates the growth of bacteria and the movement of bacteria into the egg. • Processes should be assessed for appropriateness in the washing of the egg conveyor (whether it can be high pressure washed, or hand spray and wipe).	
Egg unloaders	Cage / Barn / Free range	Regular cleaning schedule in place to prevent build-up of organic matter Must be dry before eggs come into contact with them	• Egg unloaders that come into contact with eggs should be free of extraneous matter such as dust, faeces and egg yolk. • However, this is difficult during the production day, but it is important to assess how build-up can be reduced. • Faeces are a reservoir for *Salmonella*, and yolk is an ideal growth medium for *Salmonella*. • The growth of *Salmonella* is significantly greater in the presence of water / moisture, and *Salmonella* can persist and be found in high levels in dust. • Eggs can easily break through this part of the automated collection process, and egg unloaders will need to be maintained with a regular cleaning schedule. • If wet cleaning is performed, ensure the unloaders are dry before they come into contact with eggs again. • Unloaders are inherently difficult to clean.	78, 104, 112, 173

2.2 Raw Ingredients and Feed and Water

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
FSANZ Standard 4.2.5	Cage / Barn / Free range	Producers must have an understanding of FSANZ Standard 4.2.5	• FSANZ Standard 4.2.5 clause 4 states "*An egg producer must take all reasonable measures to ensure inputs do not make the eggs unsafe or unsuitable.*" • This could be interpreted (although not necessarily as the intent) that if a producer is aware of and/or has not taken appropriate steps to manage *Salmonella* contaminated feed or water (particularly human pathogenic *Salmonella* such as Typhimurium), that the eggs from those hens may be deemed unsafe for human consumption by a state regulator. • Different state jurisdictions may interpret Standards differently. State legislation will reflect this and will need to be taken into consideration before assessing this risk.	FSANZ 4.2.5
Water	Cage / Barn / Free range	Water for hen consumption must be chlorinated or treated in a manner that significantly reduces the bacterial load, or if using bore water it should be free from pathogenic bacteria	• Continual availability of water is important to manage hen stress, as stressed hens will shed *Salmonella* in greater volumes. • Surface water can be contaminated with *Salmonella* by rodents and other animal / bird / environment factors. • Treated town water should be appropriate, but in rural areas where this is impossible, dam / tank water must be treated with chlorine or an acceptable alternative (this includes town water reservoirs on-farm). • Chlorinated water can limit *Salmonella* growth and presence. • Filtration may also be required prior to supplying to hens. • Assessment of the quality of the water will assist in	20, 52, 104, 191,194, 200, 201 National water biosecurity manual (Poultry Production)

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			determining which additives, if any, are required to the water source / lines before it reaches the hens. • Sampling water on farm is a technical issue and must be performed by a trained person. Sampling should occur more than once per year for microbial counts to ensure that an appropriate understanding of the risks for the operation from the used water source are understood and can be managed appropriately. • Ensure the hens are not able to perch above the drinking water, to limit spread of *Salmonella* through a flock by water that is contaminated with faeces.	
Water line cleanliness	Cage / Barn / Free range	Water lines and attachments should be maintained in a clean state with no organic matter build-up	• Water lines should be cleaned on a regular basis with an appropriate process, which will depend on the set up of the water lines and accompanying equipment. • Assessment of the quality of the water will assist in developing an appropriate cleaning process as the quality of the water can impact on the efficacy of the chemicals. • Organic matter can build up within the lines, especially if the quality of the water is poor and it can harbour *Salmonella*, which can also persist and grow in water. • *Salmonella* can form a biofilm in water sources. A biofilm is a protective 'coat' formed by *Salmonella* when it is in an environment that is not optimal. • A biofilm protects the *Salmonella* until it is placed under optimal conditions again and can continue to grow.	20, 52, 104, 191, 194, 201, 210 National water biosecurity manual (Poultry Production)

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Raw ingredients / feed contamination during pelleted feed production, storage and transport	Cage / Barn / Free range	Assess *Salmonella* status of raw ingredients, or the appropriate feed production, storage and transport processes should be in place to ensure feed does not become contaminated before it is received at the farm.	• Raw ingredients / pelleted feed can harbour human pathogenic *Salmonella*, and can be an important source of *Salmonella* in the food chain. • Heat-treatment process for pelleting feed is not sufficient to destroy all *Salmonella* present. However, the heat-treatment process will reduce any bacterial contamination present although it may result in the formation of a biofilm. • A biofilm is a protective 'coat' formed by *Salmonella* when it is in an environment that is not optimal, and it includes high heat. • A biofilm protects the *Salmonella* until it is placed under optimal conditions again and can continue to grow. • Some types of *Salmonella* are more resistant than others and can be recovered after being subjected to heat. • The level of *Salmonella* heat resistance is affected by the rate at which the heat is applied (i.e. the slower the temperature rise, the greater the increase in heat resistance). • All raw ingredient batches / heat-treated feed batches received on-farm must be from an approved supplier. • Supplier testing records and/or a letter outlining how the supplier manages their *Salmonella* risks should be available when requested. • Contaminated raw ingredients / feed should not be used for hen feed, however, it is inherently difficult to assess the *Salmonella* status of tonnes of ingredient / feed. • Ideally, each batch of raw ingredients / feed should be assessed for *Salmonella*, however, this is often not practical given the throughput.	21, 26, 45, 51, 59, 68, 85, 116, 126, 129, 131, 155, 175, 232 Guidelines for Food Import Control Systems (CAC/GL 47-2003)

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			• Not all *Salmonella* found in raw ingredients / feed may be transferred to the poultry and, by extension, potentially to humans. • Mash feed is a higher risk for *Salmonella* than heat-treated pelleted feed. • Feed can become re-contaminated with *Salmonella* after the heat treatment process by a number of ways, including pre-processing dust, unclean air intake during pellet cooling, out-loading bins, augers and access to insects, rodents and wild animals. • Trucks used for the transport of raw materials / feed should be cleaned on a regular basis. • Trucks transporting heat-treated feed should not be used to transport raw ingredients without an appropriate cleaning schedule in between. **Imported raw ingredients / feed can pose a risk for introducing *Salmonella* Enteritidis into the Australian layer industry.**	
Feed formulation	Cage / Barn / Free range	Feed formulation should ensure hens produce eggs with quality eggshells	• Feed formulation can affect the quality of the eggshell, as thin eggshells will crack /micro-crack more easily and facilitate the movement of bacteria into the egg. • Many factors can affect the quality of the eggshell, including hen breed, age and health status. • There are commercial feed additives that are purported to reduce the bacterial load in the feed, and others that potentially impact on the ability of various bacteria to grow and/or exist in the hen gut, but the efficacy of any feed additive to reduce *Salmonella* is affected by the *Salmonella*	125, 168, 184, 202, 230

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			status of the flock and the quality of the nutritional management. • There is limited research on the interactivity and efficacy of various in-feed additives (such as organic acids) and probiotics on the gut flora and, by extension, *Salmonella* presence and shedding. • The efficacy of organic acids to reduce *Salmonella* load in feed is affected by the type of feed. • There is evidence that *Salmonella* strains can become acid resistant in the presence of organic acids in feed. This could become a food safety issue, as interventions in the kitchen environment and the human gut, such as reduced pH, would be ineffective.	
Feed storage	Cage / Barn / Free range	Bulk feed must be covered and stored in a manner that prevents moisture and rodent / insect access, and assessed regularly for damage	• Feed should be held in a low moisture environment to prevent / limit growth of any *Salmonella*. • Rodents and insects can carry *Salmonella*, and can contaminate feed through contact and transmit *Salmonella* to hens through the feed. • Feed storage should be assessed on a regular basis (twice per year) to ensure that it is still managing the risk of water and/or rodent and insect contamination. • Feed spills outside the hen housing must be cleaned up immediately to prevent attracting wild birds and rodents.	104 AECL Biosecurity Code of Practice Animal Health Australia National Farm Biosecurity Technical Manual for Egg Production

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Feed pans / troughs etc.	Cage / Barn / Free range	Feed pans / troughs should be positioned and designed in a manner that limits access to insects / rodents and moisture	• Feed pans / troughs should be located away from the drinking water to limit water coming into contact with feed. • Assess the efficacy of the water system to prevent leaking / splashing / excess water. • Rodents and insects transmit *Salmonella* to hens via the feed, and the presence of rodents is positively correlated with the presence of *Salmonella* in a flock. • Free range feed and water should not be outside of the hen housing, and it needs to be ensured that it is covered from adverse weather and in such a way that limits contact with insects and rodents. • Feed pans / troughs must not allow the hens to perch above, as this will limit the spread of *Salmonella* through a flock by reducing faecal contamination in the feed.	70, 104 AECL Biosecurity Code of Practice Animal Health Australia National Farm Biosecurity Technical Manual for Egg Production
Feed spillage	Cage / Free range / Barn	Feed troughs / pans should be situated at a height and of a design that limits feed spillage by hens during feeding, or on purpose through scratching	• Assess the feed troughs / feed pans to ensure that hens are unable to excessively flick the feed onto the floor around the feeder. • Hens spend a considerable amount of time near the feeders and this can be one of the most faecally contaminated areas in the hen housing. • Hens will peck at feed on the ground around the pens and there is a high risk of faecal contamination of this feed, which will result in the hens ingesting faeces and, by extension, *Salmonella*. • Limiting feed spillage will also reduce rodent access to feed.	104 AECL Biosecurity Code of Practice Animal Health Australia National Farm Biosecurity Technical Manual for Egg Production

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Contamination with human effluent	Cage / Barn / Free range	Feed and water for hens must not come into contact with human effluent or improperly treated effluent water at any time	• Feed and water must not be contaminated by human sewerage / effluent water, or improperly treated sewerage water. • Humans can carry pathogenic *Salmonella* in their gut for extensive periods of time without symptoms after recovering from gastroenteritis. • Humans can also carry pathogenic *Salmonella* without exhibiting symptoms (asymptomatic). • Any effluent water (either from town treatment or on-premises storage / treatment) can be contaminated with *Salmonella* that can infect hens or contaminate feed and water. • There are different regional guidelines for microbiological limits for discharge requirements in treated effluent water that is released into water systems. • If there is effluent water or a water treatment plant in close proximity to the farm, an assessment should be conducted to determine if rodents, insects and/or wild animals that are able to come into contact with this water are able to access the farm or if the farm may have direct contact. • Improperly treated effluent water that was accessed for hen use was a significant contributor to *Salmonella* Enteritidis spread in the USA. • Therefore, it should also be considered as a potential point of entry for *Salmonella* Enteritidis into the Australian egg industry, as a high proportion of gastroenteritis in overseas travellers that return to Australia is due to *Salmonella* Enteritidis. • *Salmonella* Enteritidis is present in the Australian community and sewerage systems, and it is important to prevent reverse	104, 120, 121 AECL Biosecurity Code of Practice Animal Health Australia National Farm Biosecurity Technical Manual for Egg Production OzFoodNet Network Annual report 2010

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			zoonosis to hens.	

2.3 Egg Collection / Grading / Washing / Packing

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Grading area management	Cage / Barn / Free range	The grading area management must be of high standard, as management determines the level of *Salmonella* in / on eggs, not necessarily the production system Egg producers must understand their food safety requirements under state and federal legislation	• There are many processes during grading that can result in cross-contamination of eggs, bacterial build-up and bacterial proliferation. • It is essential that these processes are managed to a high level to ensure the risk of *Salmonella* contamination on the eggs is minimised. **Note:** Producers that grade eggs are also considered processors, and need to have an understanding of the FSANZ Standards: Standard 4.2.5 – Primary Production and Processing Standard for Eggs and Egg Product Standard 3.1.1 – Interpretation and Application exempts primary production from Standards in Chapter 3 - "*food business means a business, enterprise or activity (other than primary food production)*..." • Some states have reversed this exemption in their state legislation, in the section that relates to egg production and will require primary egg producers to adhere to additional Standards. • State jurisdictions may interpret Standards differently. State legislation will reflect this and will need to be taken into consideration.	93, 224, 241 FSANZ 4.2.5 FSANZ 3.1.1
Grading area cleanliness	Cage / Barn / Free range	The grading area should be cleaned / sanitised on a regular basis	• Bacteria can be present in the grading area on surfaces that don't come into contact with eggs (e.g. walls and floors). • High traffic areas can have a higher load of bacteria than other areas, so it is important to consider the cross-contamination implications of process and staff flow. • The grading area should be cleaned / sanitised on a regular basis to	48, 158

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			reduce the bacterial load in the grading area and to reduce the risk of cross-contamination. • The type of chemicals needed for cleaning and an appropriate cleaning schedule need to be determined (potentially once per week for egg non-contact surfaces).	
Grading area staff training	Cage / Barn / Free range	All grading area staff must be trained in food safety, with at least one regular staff responsible as a food safety manager	• Grading area staff must possess skills and knowledge in food safety and food hygiene matters commensurate with their work. This is a requirement under FSANZ Standard 4.2.5 - Primary Production and Processing Standard for Eggs and Egg Product. • State jurisdictions may interpret Standards differently. State legislation will reflect this and will need to be taken into consideration before assessing this risk. • Food safety should be considered a high priority for staff training. • Staff who are aware of the risks of *Salmonella* will be able to proactively assist in managing the risks in the grading area. • The grading area can be high risk for *Salmonella* growth due to the likely presence of yolk and organic matter from the eggs being transferred onto surfaces / equipment / machinery, and low regularity of cleaning. • There is a risk of human salmonellosis linked to eggs through improper handling and grading (and washing if performed) in the grading area, as *Salmonella* can proliferate to levels that can cause human illness. • This is a risk to business reputation and profitability, hence the need to encourage staff to assess their tasks for areas that could be continually improved in terms of food safety. • Humans can carry pathogenic *Salmonella* in their gut and may be	104, 141, 144, 172 FSANZ 4.2.5

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			able to contaminate eggs (or grading equipment), and can be symptomatic or asymptomatic. • This is particularly important for staff returning from international travel, due to the risk of *Salmonella* Enteritidis. • Any staff member who has an intestinal upset must not have access to the grading area. • Maintain a regular toilet cleaning schedule, and ensure both hand washing and hand sanitising facilities are available and used.	
Best before date of 42 days	Cage / Barn / Free range	The best before date is a function of egg quality not food safety	• The AECL code of practice indicates that the 'best before' date should be a '*period of six weeks or less from the date of pack, only if the eggs are continually kept under optimal temperature conditions*'. • There are no legal requirements for eggs to have a best before date of 42 days, unless required by a customer. It is an industry set standard that estimates the function of egg quality not egg safety. • The best before date is '*from the date of pack*' instead of '*from the estimated date of lay*'. This may have negative implications for food safety as eggs can be repacked and be contaminated by *Salmonella* at any stages through-chain and therefore the best before date may not accurately represent the quality or safety of the egg. • *Salmonella* has been shown not to persist on eggshells past four weeks, which may mean that the eggshell of old eggs has less *Salmonella* present on the shell than new eggs (assuming recontamination has not occurred through-chain and the eggs have been stored appropriately). • The antibacterial efficacy of the albumin reduces over time as does the quality of the internal contents due to water loss and protein degradation, which will increase the risk of *Salmonella* causing human illness if the *Salmonella* has been able to contaminate the	46, 73, 141, 184 AECL Code of practice for shell egg production, grading, packing and distribution Recommendation: Update the AECL code of practice to indicate best before date as 6 weeks or less from *estimated date of lay* rather than *date of pack*.

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			internal contents and migrate to the yolk (referred to as the 'Yolk Mean Time'). Yolk is an ideal growth medium for *Salmonella*.	
Egg usage and handling by customers	Cage / Barn / Free range	Producers should understand how their eggs will be used by their customers	• Producers may need to assess the risk to their business if their customers produce high risk egg products (such as raw egg sauces) as it is highly likely that the producer will be investigated by the relevant government authorities in the event of a salmonellosis outbreak in these food service outlets, even if the producer has taken all reasonable measures to manage their *Salmonella* risk. • Producers are also able to provide advice to their customers (in the form of pamphlets, etc.) regarding proper egg handling techniques (including storage), if they are concerned that the handling of eggs by their customers puts them at high risk of foodborne illness outbreaks.	241 AECL Egg Safety Flyer Recommendation: Update the AECL egg safety flyer as some of the advice requires adjustment (e.g. hands should be washed after handling ANY egg).
Egg recalling	Cage / Barn / Free range	A process must be in place that can be followed in the result of an egg recall	• Producers must have a withdrawal / recall plan, in the event of foodborne illness outbreak(s) linked to their eggs. • This process aligns with the requirements under FSANZ Standard 4.2.5 - Primary Production and Processing Standard for Eggs and Egg Product, clause 10, which states "*an egg producer must have a system to identify to whom eggs or egg pulp is sold or supplied*." • State jurisdictions may interpret Standards differently. State legislation will reflect this, and will need to be taken into consideration before assessing this risk.	239, 241 FSANZ 4.2.5

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Egg packaging	Cage / Barn / Free range	Protect the eggs from expected / known traumas associated with egg transport and storage	• The packaging material (including the egg carton) should protect the eggs from bacteria and other pathogens, natural predators, loss of moisture, tainting, temperatures that cause deterioration, and possible mechanical stressors while being handled, stored or transported. • Cracked eggs cannot be sold under FSANZ Standard 2.2.2. Some stakeholders may interpret the standard as meaning that there are no cracked eggs at time of receipt of delivery, not the time they left the grading area. • Some customers may reject shipments with cracked eggs, as they are prohibited to on sell them, hence it is at a cost to the producer. • Assessment may include the retail packaging for storage onto a shelf, to minimise handling where the eggs will be stored for retail purchase, bulk purchase and/or storage.	3, 199 FSANZ 2.2.2
Insects and rodents	Cage / Barn / Free range	Minimise insect and rodent load near grading area Have an adequate and regularly monitored pest control program in place Replace baits as required	• Minimising insects (including flies, beetles and cockroaches) and rodents (including pet rodents) from inside the grading area will minimise the risk of *Salmonella* contamination on the eggs, grading equipment and packaging. • Ensure that any rodent bait stations are monitored and re-baited regularly. • The presence of rodents is positively correlated with the presence of *Salmonella* in a flock.	58, 70, 80, 94, 104, 123, 145, 185, 188, 219, 225 AECL Final Report DAQ-244J *Investigations into the management of the darkling beetle*

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Animals	Cage / Barn / Free range	Secure grading area from unwanted access to animals	• Various animals are carriers for *Salmonella* including chickens, turkeys, dogs, cats, horses, guinea pigs, pigs, cattle, sheep, wild birds (e.g. ducks, pigeons, finches and sparrows) and other wild animals (such as foxes). • Animals can contaminate the grading area / equipment, which can then cross-contaminate eggs. • There is minimal research demonstrating specific carriers for specific types of *Salmonella*, as it is suspected that *Salmonella* is often not host-specific.	20, 28, 83, 103, 104, 121, 151, 165, 174, 203, 231
Temperature and humidity control on the grading floor	Cage / Barn / Free range	Ensure temperature and humidity are controlled in the grading area so that environmental fluctuations during seasons do not impact on the eggs	• Control of temperature and humidity can help control the growth of bacteria and any moisture caused by condensation through inappropriate humidity control. • Moisture on eggs can promote bacterial growth and facilitate the movement of bacteria into the internal contents. • Grading areas that cannot / do not have an electronically controlled environment should be assessed for any effects on eggs during varying temperature / humidity situations, such as would occur through seasonal variation throughout the year. • Electronically controlled environments may need to be assessed through seasonal variations as the layout of the grading area could impact on the efficacy of the environmental control. • The appropriate humidity will depend on the temperature and climate conditions. Humidity of 65-70% is considered acceptable. • Survival of *Salmonella* is greater at lower humidity than at high humidity.	53, 104, 148, 173

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Time between when the egg is laid and collection	Free range / Barn	Eggs should be collected at least once every 24 hours More frequent collection (e.g. every 2 hours during peak time of lay during the day) if hens are laying floor eggs	• Extended contact with faeces / extraneous matter, including egg internal contents from broken eggs, increases the risk of *Salmonella* on the eggshell and the facilitation of bacteria through the eggshell. • Faeces are a reservoir for *Salmonella*, and yolk is the ideal growth medium. • Bacteria present will continue to grow before the egg enters cold-chain storage (*Salmonella* will still grow at 15°C), if organic matter is present. • Regular egg collection will prevent a build-up of the number of eggs, which can result in breakages and bacterial growth in the egg internal contents (especially the yolk). • Regular collection also aids in egg recovery rates and increased profitability for producers.	104, 111, 148, 180, 209
Egg collection containers	Free range / Barn	Any containers used to collect barn / free range and/or floor eggs should be cleaned and sanitised at least once per day	• Egg collection containers come into contact with eggs and should not have extraneous matter, including faeces and egg yolk. • Faeces are a reservoir for *Salmonella*, and yolk is the ideal growth medium. • Any contamination present in collection containers can contaminate eggs that come into contact with it in subsequent collections. • Bacterial build-up can occur if organic matter is present, which can easily contaminate eggs with a larger load of bacteria than was already present on the surface of the eggs. • Visible organic matter can be brushed off the containers after each collection or at least once per day to reduce organic matter load. • At minimum, a wet cleaning / sanitising / drying process should be carried out at least once per day to prevent bacterial build-up.	78, 104, 112, 148, 160, 173

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			• Containers must be dry before eggs come into contact with them, as water / moisture can facilitate bacterial growth and the movement of bacteria into the egg. • Consideration should be given to cleaning the equipment that is used to transport the eggs from the hens to the grading floor as these transporters have been shown to have high levels of bacteria present.	
Wet floor eggs / dry floor eggs	Free range / Barn	Wet floor eggs should be discarded Dry floor eggs should be processed separately to non-floor eggs, and should be cleaned	• Floor eggs have a significantly higher risk for bacterial contamination on and in eggs because they come into contact with dirt / dust / faeces / moisture at a greater rate than eggs laid in controlled areas. • Moisture / water facilitates the growth of *Salmonella* and the penetration of *Salmonella* into the egg, hence wet floor eggs are of considerable concern as a high proportion of eggs that come into contact with a wet environment is likely to be internally contaminated, and therefore should not be graded for human consumption. • Dry floor eggs should be processed separately to non-floor eggs to limit the risk of cross-contamination to clean eggs, and should be cleaned through a closely monitored, verified, washing and/or sanitisation process to remove the additional contamination that is present (whether visible or not). • Some breeds of hens may be at higher risk of laying eggs on the floor than other breeds. • Diffusion into the egg can occur due to differences in density / pressure, because of temperature differences between the internal egg and the outer environment. • Any external application to the egg must be at a temperature higher than the internal temperature of the egg (including the eggshell) so	14, 35, 38, 44, 104, 112, 148, 173, 180, 189, 193, 205, 209, 215 Recommendation: Assess whether it is feasible to update FSANZ Standard 4.2.5 and 2.2.2 to prohibit wet floor eggs for human consumption as shell eggs (even after washing to remove dirt).

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			that diffusion is from inside the egg to outside. • If an egg is laid into a wet environment or comes into contact with water that is a lower temperature of the internal contents (an egg is laid at approximately 41°C), the result is diffusion from outside to inside the egg, i.e. transfer of anything on the surface of the egg (including bacteria like *Salmonella*) into the egg. • Research into *Salmonella* penetration into eggs has found that it is easier to artificially contaminate eggs with a wet solution of faeces containing *Salmonella* as opposed to a dry faecal sample. • The rate of bacterial penetration into the egg will depend on a number of variables including hen age, shell quality (which can be affected by age, health status and diet), hen breed and *Salmonella* type. • There is a considerable amount of research into *Salmonella* penetration in hatching eggs, most of which is applicable to table eggs (i.e. that risk factors for bacterial penetration will be the same, etc.). • More research is required into the efficacy of specific interventions for reducing floor eggs, however, there would be a number of variables that would affect any research-based understanding of efficacy (such as flock management, climate / weather changes).	
Plastic fillers	Cage / Barn / Free range	Plastic fillers must be cleaned and sanitised after every use	• Plastic fillers should be cleaned and sanitised appropriately after every use. • *Salmonella* can form a biofilm on plastic as there are often no organic components to promote bacterial growth (a biofilm is a bacterial protective 'coat' when it is in unfavourable conditions). • Organic material should not be present on plastic fillers before eggs	211, 233

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			are placed into them. • Some research indicates that not all cleaning and sanitising processes may be able to remove bacterial biofilms. • Other pathogens can also be present on plastic fillers and therefore the fillers can be a vehicle for transportation of pathogens from farm to farm or flock to flock. • Plastic fillers are lower risk than cardboard for *Salmonella* contamination.	
Cardboard fillers	Cage / Barn / Free range	Cardboard fillers should be discarded after a single use	• Any bacteria present on the eggs can be transferred to any surface that the egg comes into contact with. • Reusing cardboard fillers can result in the transfer of bacteria onto new eggs present in the filler. • Cardboard is absorbent and able to retain bacteria and other organic matter. • If water / moisture is present, bacteria can grow and can contaminate eggs that it comes into contact with. • No peer-reviewed article could be found for *Salmonella* specifically on cardboard, however, there are articles describing other bacteria presence and growth on cardboard, and there is anecdotal evidence to support single use of cardboard fillers only.	104

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Presence of yolk	Cage / Barn / Free range	Reduce the presence of yolk, particularly on equipment that has regular / sustained contact with eggs (e.g. conveyors, tables) Clean and sanitise	• *Salmonella* grows rapidly in egg yolk as it is an excellent growth medium and the growth rate of bacteria in yolk alone is significantly greater than whole eggs at all storage temperatures that have been investigated. • 10^8-10^9 CFU/mL of *Salmonella* was achieved three days at 15°C, 26 hours at 22°C, and 9 to 10 hours at 37°C. • The dose of *Salmonella* required to cause human illness can be below 10^3 CFU. • Egg yolk should not be present on equipment that is in regular contact with eggs such as conveyors, sorting tables and grading equipment. • Antibodies present in the yolk have been shown to have little effect on the growth of some types of *Salmonella*, including Typhimurium, but other studies have indicated there may be some inhibitory effect. • The efficacy of antibodies may be affected by a number of variables.	17, 31, 73, 74, 104, 141, 195, 220
Equipment / surfaces in contact with eggs	Cage / Barn / Free range	All equipment that comes into contact with eggs should be cleaned and sanitised regularly (e.g. once per week) Limit visible yolk	• Bacteria present on the eggshell can transfer to equipment and surfaces that are in contact with the eggshell and build up over time. If yolk is present then bacteria will multiply and build up faster. • Equipment in regular contact with eggs should be cleaned and sanitised on a regular basis. • Appropriate practice may include dry cleaning and sanitisation of equipment at the end of every grading day, and complete wet washing and sanitisation once per week. • *Salmonella* can persist on a number of different materials including plastic and stainless steel.	48, 115, 152, 157, 161, 233

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Equipment that has been in contact with unwashed eggs coming into contact with washed eggs	Cage / Barn / Free range	Equipment that comes into contact with unwashed eggs must not be used for washed eggs	• Eggs that have been washed must not come into contact with equipment that has come into contact with unwashed eggs as the washed eggs can become easily contaminated. • The cuticle of the eggs, and some part of the eggshell, may be removed during the washing process so that the eggs are more vulnerable to shell contamination and bacterial penetration into the egg after washing than before washing.	19, 48, 77, 115, 157, 161
Leaking and grossly dirty eggs present through entire grading process	Cage / Barn / Free range	Cracked or dirty eggs must be removed from whole egg processing as soon as possible	• A requirement under FSANZ Standard 2.2.2 is that visibly cracked (visible, or visible by candling or other equivalent methods) and dirty eggs (both defined as 'unacceptable' eggs) are prohibited from sale. • FSANZ Standard 4.2.5 (clause 11) also enforces this by stating that "*An egg producer must not sell or supply eggs or egg pulp for human consumption if it knows, ought to reasonably know or to reasonably suspect, that the eggs are unacceptable*." • Leaking and grossly dirty eggs can contaminate other eggs and surfaces / equipment / belts with egg internal contents and any bacteria / matter on the surface of the eggs. • Faeces and egg yolk are ideal growth medium for *Salmonella*, and can act as a reservoir for bacteria. • Leaking and grossly dirty eggs should be removed for separate processing as soon as possible from the grading line to minimise *Salmonella* contamination through the entire grading process and other eggs that contact those contaminated surfaces. • Ideally, leaking and grossly dirty eggs should be removed prior to the washing machine as the contents of eggs can interfere with the efficacy of egg washing chemicals (especially important if the wash	19, 27, 48, 104, 115, 157, 161, 222, 223, 228 FSANZ 4.2.5 FSANZ 2.2.2

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			water is reused). • There is a significant risk that grossly dirty eggs are already internally contaminated by the time they reach the grading area and should be removed as soon as possible. • Two research papers from the 1960s, investigating *Salmonella* penetration in cracked eggs, were the only peer-reviewed research that could be found on the topic. • Both investigations demonstrated that cracked eggs alone were not of greater risk for spoilage than normal eggs. • Unwashed cracked eggs and cracked eggs washed in clean water were apparently as resistant to spoilage as uncracked eggs receiving the same treatments. • Inappropriate management of washing processes substantially increased spoilage of cracked eggs compared to normal eggs. • This type of research would need to be repeated in the current commercial environment. No comprehensive study could be found that investigated what potential quantitative increase in risk of *Salmonella* contamination cracked eggs pose. This could be because it is difficult to define a cracked egg and *Salmonella* can easily transfer through intact shells. However, a number of industry risk assessments have concluded that cracked eggs pose a high risk of human salmonellosis if consumed.	
Automatic crack detector	Cage / Barn / Free range	Equipment must be checked regularly and cleaned appropriately	• Visibly cracked eggs are prohibited from sale under FSANZ Standard 2.2.2 "*cracked egg means an egg which has a cracked shell which is visible, or visible by candling or other equivalent methods, and includes a broken egg.*" • The crack detection method must not compromise food safety by	27, 113, 222, 223, 228 FSANZ 2.2.2

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			introducing *Salmonella* onto the eggs. • The sensitivity of automatic crack detectors can be reduced or increased, as the accuracy of crack detection will depend on a number of variables (e.g. different shell thicknesses due to age, health status). • Automatic crack detectors that come into contact with the egg are highly susceptible to contamination from bacteria on the outside of the eggshell but also from egg contents from broken / cracked eggs. This will lead to contamination of every subsequent egg that the detector comes into contact with. • Crack detectors are delicate instruments and great care must be taken to clean them. Also, the manufacturer's instructions may advise against the application of wet cleaning chemicals. • There are different methodologies for automatic crack detection and minimal research could be found to compare for efficacy. • Two research papers from the 1960s, investigating *Salmonella* penetration in cracked eggs, were the only peer-reviewed research that could be found on the topic. • Both investigations demonstrated that cracked eggs alone were not of greater risk for spoilage than normal eggs. • Unwashed cracked eggs and cracked eggs washed in clean water were apparently as resistant to spoilage as uncracked eggs receiving the same treatments. However, inappropriate management of washing processes substantially increased spoilage of cracked eggs compared to normal eggs. • This type of research would need to be repeated in the current commercial environment. No comprehensive study could be found that investigated what potential quantitative increase in risk of *Salmonella* contamination cracked eggs pose. This could be	

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			because it is difficult to define a cracked egg and *Salmonella* can easily transfer through intact shells. However, a number of industry risk assessments have concluded that cracked eggs pose a high risk of human salmonellosis if consumed.	
Manual crack / default egg detection	Cage / Barn / Free range	Must be performed only by an operator with acceptable training	• Visibly cracked eggs are prohibited from sale under FSANZ Standard 2.2.2 "*cracked egg means an egg which has a cracked shell which is visible, or visible by candling or other equivalent methods, and includes a broken egg.*" • Manual candlers should wash their hands before and after candling, and before they handle anything else. • Access to antibacterial gel may be appropriate for operators during candling. • Operators should be rotated regularly to prevent fatigue, which will reduce the ability of the operator to detect cracks. • How regularly the rotation needs to occur will depend on a number of variables including operator fatigue time, sight and concentration span. • The operator should take care with touching eggs as this may cause cross-contamination if a dirty / cracked egg is touched before a clean egg. • It is not feasible to expect the operator to clean their hands after each touch of dirty eggs. However, hands should be washed appropriately after handling leaking eggs. • Ensure adequate hand washing facilities are present in a convenient location for all grading staff. • The speed of the eggs moving through the crack / fault detection	27, 113, 222, 223, 228 FSANZ 2.2.2 Poultry CRC Egg Grab'n'Grade game – a simulator is also available for training

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			process should be adjusted according to the ability of the candler. • There are a number of ways to manually candle eggs but minimal research could be found to compare manual candling methods. • Two research papers from 1960s, investigating *Salmonella* penetration in cracked eggs, were the only peer-reviewed research that could be found on the topic. • Both investigations demonstrated that cracked eggs alone were not of greater risk for spoilage than normal eggs. • Unwashed cracked eggs and cracked eggs washed in clean water were apparently as resistant to spoilage as uncracked eggs receiving the same treatments. • However, inappropriate management of washing processes substantially increased spoilage of cracked eggs compared to normal eggs. • This type of research would need to be repeated in the current commercial environment. No comprehensive study could be found that investigated what potential quantitative increase in risk of *Salmonella* contamination cracked eggs pose. This could be because it is difficult to define a cracked egg and *Salmonella* can easily transfer through intact shells. However, a number of industry risk assessments have concluded that cracked eggs pose a high risk of human salmonellosis if consumed.	
Thermal cracking	Cage / Barn / Free range	Prewash eggs with a rinse that is below the first wash solution temperature	• The eggshell can micro-crack / crack / fracture / break (in a way that is potentially unobservable) if it is exposed to a sudden change in temperature (cold to hot, or hot to cold). • Visibly cracked eggs are prohibited from sale under FSANZ Standard 2.2.2 "*cracked egg means an egg which has a cracked shell which is visible, or visible by candling or other equivalent*	33, 153 FSANZ 2.2.2

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			methods, and includes a broken egg." • Evidence suggests that a temperature differential alone will not be sufficient to crack an intact eggshell, however, it is suspected that if a hairline fracture or unobservable crack is present, then a substantial temperature change (e.g. 20°C) can potentially create larger, visible, cracks. Further research is required for confirmation. • Thermal cracking is most applicable to eggs that are stored in a cool room before washing / processing, or have chilled excessively in low ambient temperatures. • The temperature of the eggshell (not the internal contents) should be gradually increased to the washing temperature, which can be achieved through a pre-rinse step. • A pre-rinse will also help to initially loosen extraneous matter before the washing process, which can improve the efficacy of the washing solution step.	
Extraneous matter on the surface of the egg	Cage / Barn / Free range	Organic matter must be removed from the outside of the egg	• Under FSANZ and state legislation, dirty eggs are prohibited from sale on the basis of food safety, and to limit the load of *Salmonella* (and other bacteria) that is present on the eggs before they enter the supply chain. • Faeces are a reservoir for *Salmonella* and if *Salmonella* is in contact with the surface of an egg for any length of time the risk increases that the bacteria may penetrate into the egg. The risk is increased if there is water / moisture present, especially if the internal egg temperature is higher than that of the ambient temperature / moisture and therefore is reducing and setting up a negative pressure. • *Salmonella* is motile and can move itself into the egg. Inappropriate grading / washing practices can assist the movement of bacteria into	104, 143, 193, 209, 215 FSANZ 2.2.2 FSANZ 4.2.5

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			the egg. • Environmental enrichment can increase the invasiveness of *Salmonella* to the human gut and promote growth of the bacteria, which can also contaminate any surface the egg comes into contact with. • Environmental enrichment differs between the farm and kitchen environments, so it is important that eggs are as bacterially clean as they can be before they enter an environment that promotes and facilitates *Salmonella* growth. • Dirty eggs should be removed from the grading process before any washing process, as there is a high chance that the eggs are already contaminated internally.	
Dry egg cleaning method	Cage / Barn / Free range	Dry paper towels or dry clean cloths can be used to remove extraneous matter, however, they need to be changed frequently (i.e. if they become wet, dirtied with faeces, etc.) Equipment in contact with eggs cleaned and sanitised daily	• FSANZ Standard 4.2.5 defined a dirty egg as "*dirty egg means an egg that has visible faeces, soil or other matter on it*." • Dry cleaning method can be used to remove small marks of dirt or faeces, however, it is strongly recommended that dirty eggs are not recovered for A grade eggs as there is a significant risk that these eggs are already internally contaminated. • Do not use a wet cloth as the moisture from this will encourage the growth of bacteria and can be transferred from the cloth to hands and other eggs or surfaces, and will facilitate the movement of bacteria into the egg. • Whatever is being used to remove the extraneous matter should be changed regularly so that bacterial contamination is not spread across to eggs or equipment, etc. that may have been uncontaminated with *Salmonella*. • Cloths should be dry at all times and never allowed to be wet or	15, 104, 119, 193, 209 FSANZ 4.2.5 AECL Code of practice for the manufacture of egg products

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			overly dirty (discard or wash / dry appropriately if overly dirty). • UV application may also help reduce bacterial contamination, however, UV may not be able to destroy bacteria present in visible (organic) matter on the egg. • There are a number of variables that will impact on the efficacy of UV to reduce *Salmonella* load. • Oiling or post-washing application onto the eggs does not seem to be widely performed in the Australian industry. • Post-washing applications such as oil or other food grade waxes have varying efficacies on maintaining egg quality (through prevention of water loss) and reducing porosity of the eggshell, which can help reduce the risk of *Salmonella* entering the egg. • There may be scope to use a sanitiser as part of this process. • No research could be found that investigated the efficacy of dry cleaning with wet washing.	
Egg washing	Cage / Barn / Free range	Washing to remove extraneous matter must be performed carefully with appropriate analysis of the hazards present for the process Process must be validated to reduce bacterial load	• Washing and sanitising puts the eggs into contact with water, which increases the risks of cross-contamination of clean eggs with dirty water, therefore allowing bacterial diffusion into the egg and facilitating the movement of motile bacteria, such as *Salmonella*, into the eggs. • Not wet washing is better than poor wet washing, therefore washing must be done with extreme care and thorough understanding of the risks. • Egg washing is not required in Australia but the sale of dirty eggs is prohibited under FSANZ Standard 2.2.2.	15, 29, 59, 63, 77, 104, 107, 119, 157, 159, 161, 189, 198, 236 FSANZ 2.2.2 Recommendation: Assess whether producers should

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			• The washing process should be validated to effectively reduce the bacterial load on the surface of the egg, as visually clean eggs can still be highly contaminated with *Salmonella.* • Validation includes automated washing machines as these machines are designed to produce a high recovery rate of first grade eggs, not necessarily to reduce the load of bacteria on the surface of the egg. • The chemicals used for the process should be appropriate and be carefully monitored to ensure chemicals are active and at appropriate concentrations. • Washing in water alone can reduce the bacterial load on the eggshell but is not as effective as appropriate washing with appropriate chemicals. • There is some evidence that egg washing can be performed at cooler temperatures without impacting on the microbiological quality of the eggs, provided that the wash solution temperature remains above the internal temperature of the eggs. • UV application may also help reduce bacterial contamination, however, it may not be able to destroy bacteria present in visible matter on the egg. • There are a number of variables that will impact on the efficacy of UV to reduce *Salmonella* load. • Post-washing applications such as oil or other food grade waxes have varying efficacies on maintaining egg quality (through prevention of water loss) and reducing porosity of the eggshell, which can help reduce the risk of *Salmonella* entering the egg, but must be verified appropriate before use.	be registered or licensed to wash eggs.

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Contaminated egg washing / grading equipment	Cage / Barn / Free range	After every production period, equipment must be cleaned and sanitised on a regular basis	• If the chemicals are adequate and verified then the washing process is self-cleaning. • Reuse tanks must be cleaned after every production period to prevent bacterial and organic matter build-up. • A sanitiser that is fit for purpose can be applied to the equipment after cleaning and drying of the equipment. • Calcium from the eggshells can block the sprays and interfere with the wash chemicals, as can the internal contents of eggs if broken eggs are put through the washing process. • Bacteria present on the eggshell can transfer to equipment and surfaces that are in contact with the eggshell and build up over time. If yolk is present then bacteria will multiply and build up faster. • Equipment in regular contact with eggs should be cleaned and sanitised on a regular basis. • Appropriate practice may include dry cleaning and sanitisation of equipment at the end of every grading day and complete wet washing and sanitisation once per week. *Salmonella* can persist on a number of different materials including plastic and stainless steel.	48, 115, 152, 157, 161, 233
Contaminated suction cups / vacuum loaders in automated grading / washing machines	Cage / Barn / Free range	Equipment must be cleaned on a regular basis	• The suction cups / vacuum loaders that transfer eggs throughout the grading process should be maintained in a clean state. • If inadequate pre-grading processes are in place the possibility remains that cracked / broken / dirty eggs will be present, which can contaminate the suction cups, which can then contaminate other eggs that may have been free of *Salmonella* or may increase the load of *Salmonella* present on the eggs. • Suction cups have been reported as being an ideal location to test for the presence of *Salmonella* on a grading floor, as they can have	48, 108, 114, 115

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			substantial bacterial build-up if not cleaned regularly. • An appropriate cleaning schedule may include replacing suction cups during egg processing with a spare set of suction cups and putting the dirty suction cups through a process to clean and dry them before they are used again.	
Inappropriate wash cycle	Cage / Barn / Free range	The wash cycle should be validated for reducing bacterial load on the eggs, and control parameters should be verified regularly during production	• Washing the eggs may damage / remove the egg's protective cuticle, which will increase the risk of bacterial transfer into the egg. • Wash water temperatures are critical to prevent extraneous matter from being diffused into the eggs, as the internal temperature of the egg should not be allowed to reach the temperature of the wash water, which should be higher than the internal temperature of the egg. • The washing process should be validated to effectively reduce the bacterial load on the surface of the egg as visually clean eggs can be highly contaminated with *Salmonella.* • The chemicals used for the process should be appropriate and carefully monitored (verified) to ensure that chemicals are active and at appropriate concentrations. • Eggs must never be left to stand in any solution, as they will immediately start to equilibrate to the temperature of the solution. • The eggshell equilibrates almost immediately, however, it takes longer for the internal contents to equilibrate and the rate of equilibration will depend on the temperature differential.	19, 56, 77, 186, 189, 227
Inadequate / incompatible egg washing chemicals	Cage / Barn / Free range	Must be appropriate for purpose and tested regularly for activity	• Wash chemicals can be diluted by reusing water, washing sanitiser into reused wash water and other water recycling methods. • The level of chemical should be checked regularly (e.g. every 30	10, 63, 107, 198

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			minutes) to verify that the washing process is still effective. • Chemical levels can be assessed using simple methods such as chemical test strips. • pH level alone is not an accurate measure of chemical activity or presence, as other factors such as calcium from eggshells or other organic matter (faeces) can impact pH. • The control of temperature and pH alone was found to be insufficient to control bacteria in the wash water. Based on a recommended temperature of >40°C and pH >10, the use of a chlorinated alkaline detergent with a minimum total available chlorine concentration of 0.45mg/l was recommended by Bartlett et al 1993.	
Inadequate temperatures during egg washing	Cage / Barn / Free range	The temperature of each solution must be higher than the previous solution in the washer and measured at the egg surface (includes wash solution, sanitiser)	• Diffusion across the eggshell occurs because of differences in density / pressure due to heat application. • The higher the temperature, the lower the density / pressure (think water – steam), and diffusion occurs from areas of high density / pressure to low density / pressure. • Any external application to the egg must be at a temperature higher than the internal temperature of the egg so that diffusion is from inside the egg (high density) to outside (low density). • Assess the internal temperature of the eggs going into the washing process so that there is an understanding of the temperature start point, as the internal temperature may not reflect the storage temperature. • The temperature of each solution applied to the eggs must be higher than the temperature of the process before, and measured at the egg surface. • In automated systems it is important to adjust the temperature of the	56, 227

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			wash solution in the tank to a level that takes into account the loss of temperature during spraying / application on the eggs. • The temperature at the egg surface can be up to 10°C below the temperature in the tank. • Eggs must never be left to stand in any solution, as they will immediately start to equilibrate to the temperature of the solution. • The eggshell equilibrates almost immediately, however, it takes longer for the internal contents to equilibrate and the rate of equilibration will depend on the temperature differential.	
Damaged and dirty brushes in the washing process	Cage / Barn / Free range	Assessed at the start of each run day to ensure equipment is effective and clean	• Brushes can wear and tear, and lose their ability to agitate the wash solution and brush the eggs. • Continual use will shape the brushes to the shape of the eggs, which will limit the efficacy of the brushes to remove debris from the eggs. • The brushes should also be maintained in a clean state as broken eggs through the washing machine can leave egg material on the brushes, which can coagulate with the heat of the wash solution.	115
Damaged / blocked / unaligned egg washing machine nozzles	Cage / Barn / Free range	Equipment should be checked regularly that it is working effectively	• Nozzles can be damaged or placed incorrectly, leading to sprays that do not produce a total coverage of the eggs. • This may result in ineffective washing processes, which can be a significant risk. • The nozzles can also become blocked, so they should be checked before every processing run that they are still effective, and aligned appropriately.	63

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Use of a terminal sanitiser on eggs and equipment	Cage / Barn / Free range	It is unclear whether terminal sanitisers are approved for use on eggs in Australia	• Washing removes the protective cuticle and part of the eggshell and leaves the shell vulnerable to re-contamination through the supply chain. • A terminal sanitiser is one that is not washed off after application and has a residual sanitiser effect, which is more efficacious as it is left on the egg. Egg internal temperatures must be below the sanitiser temperature so there is no risk that it can be drawn into the egg under negative pressure. • Anecdotal evidence and IP-protected research indicates that the residual effect of registered sanitisers in the USA is not longer than 10 minutes. • The USA has approved a number of terminal sanitisers for use on eggshells, however there exists no similar process in Australia (that reflects the roles of the FDA and EPA in the USA) through which these types of chemicals can be approved. • Terminal sanitisers can be used to sanitise equipment in contact with eggs on a regular basis (e.g. at the end of every processing run), in between scheduled thorough cleaning. This will reduce the bacterial load on equipment, especially equipment that comes into contact with eggs. • Terminal sanitisers are used routinely (including in Australia and are approved by APVMA for use, e.g. Ambicide) to increase the hatch rate in hatching eggs, as they are proven to reduce bacterial load and therefore bacterial penetration into the hatching eggs.	19, 25, 77, 104, 176, 198, 226, 236 EPA Guidance for Table Egg Sanitisers List of sanitisers approved for table egg use in USA – Q3, Q4 and Q6 in the list located in the box Recommendation: Terminal sanitisers allowed for use in Australian egg production to provide clarity to producers already undertaking this practice and to align with the USA. FSANZ to comment?

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Washing and grading machine / process stoppages / pauses	Cage / Barn / Free range	There should be interconnected stopping mechanisms for the washing systems to ensure that, if the whole process needs to pause or stop, the eggs are not left stalled in the washing machine in prolonged contact with water	• Procedures should be in place to ensure that eggs are held in the wash system for a period of time that does not equilibrate the temperature of the internal contents of the eggs with any wash solution. • Eggs may need to be discarded if they are subjected to a single solution for too long as the internal contents may become contaminated through diffusion into the egg. Investigate what is an acceptable time for each situation, as each operation is different. • Assess the internal temperatures of the eggs held in a heated process at different lengths of time to determine the parameters for the process. • Clear the grading floor / washing / grading machine of eggs prior to breaks or during breakdowns, so that the eggs can be maintained at an appropriate temperature and no eggs are in contact with water / solution / heat and drying or contaminated surfaces for a prolonged period. • There are too many variables to assess what may be an appropriate industry recommendation, but potentially some research could be undertaken to assess the priority variables (internal egg temperature, stage of washing, etc.).	56, 115, 227
Repeated cycling of dirty eggs through the wash process	Cage / Barn / Free range	Dirty eggs should not be allowed to continually cycle through the wash process	• If the wash solution is not under control with regard to the levels of active solution, washing and sanitising puts the eggs into contact with water which significantly increases the risks of cross-contamination of clean eggs with dirty water, for bacterial diffusion into the egg, and facilitating the movement of *Salmonella* into the eggs. • No wet washing is better than poor wet washing. Therefore, washing must be done with extreme care and thorough understanding of the	19, 63, 77, 159, 209, 228

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			risks. • Egg washing is not required in Australia but the sale of dirty eggs is prohibited under FSANZ Standard 2.2.2. • The washing process should be validated that it effectively reduces the bacterial load on the surface of the egg as visually clean eggs can still be highly contaminated with *Salmonella.* • Validation includes automated washing machines as these machines are designed to produce a high recovery rate of first grade eggs, not necessarily to reduce the load of bacteria on the surface of the egg. • If the wash process cannot remove visible organic matter from the surface of the egg then either the process is not adequate or the egg may be beyond cleaning for human consumption. • The maximum number of times the egg should be allowed to cycle will depend on the washing process efficacy and set-up, however, given that internal contamination is highly likely in dirty eggs, it could be feasible to suggest that eggs are not cycled at all. • It is recommended that dirty eggs be removed from processing prior to the washing process. • Further, continued cycling through a washing process will continue to damage the eggshell and remove the protective cuticle. • Dirty eggs that are continually allowed to cycle through the wash process will increase the risk of those eggs contaminating machinery, equipment and other eggs. • The more times an egg is cycled through the wash process the higher the internal temperature of the eggs will be, as the internal temperature will rise to equilibrate with the wash solution temperatures.	

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			• If the internal temperature becomes equal to or exceeds the temperature of the wash solutions then diffusion will cause matter (including *Salmonella*) to diffuse into the egg. • The system will need to be monitored and verified to understand how long it takes for the eggs that are being cycled through the wash process to reach the temperature of the wash solution so that the eggs can be removed before the internal contents of the eggs reach that temperature. • Removing eggs that are still visually contaminated after a single cycle through the washing process, for use in pulp and pasteurisation should be considered as an appropriate intervention.	
Inadequate and/or unclean egg dryers	Cage / Barn / Free range	Regular assessment of positioning, and ensuring that air intake is uncontaminated	• Eggs must be dried after washing as any water on the surface of the egg will facilitate the movement of bacteria into the egg and the growth of bacteria. • The dryer components can be damaged or placed incorrectly, which may reduce the efficacy of drying. • The air intake should not be contaminated or drawn from a contaminated area. • The egg dryer can be a source of airborne bacteria, which is of particular concern for eggs that have just been washed, as washing increases the susceptibility of the eggs to bacterial contamination and (external and internal). • A filtered air intake may be required. The temperature of the dryer should maximise the drying capabilities of the air by raising the temperature or reducing the humidity, or both. • The temperature of the air will affect the efficacy of drying and it should be higher than the temperature of the last process to ensure	77, 104, 166

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			diffusion into the egg does not occur. • Ensure that the dryer is also able to dry the conveyor, as a wet conveyor can rewet the eggs after drying. • There should be regular assessment of this process to ensure that it is effective to dry the eggs without contaminating them.	
Egg stamping	Cage / Barn / Free range	Eggs for retail sale or for sale to a caterer must be individually marked with the producer's or processor's unique identification	• Egg stamping can assist in the traceability of eggs during a human foodborne illness outbreak and is required under FSANZ Standard 2.2.2. • Egg stamping should be performed after any washing process to ensure that the stamp is legible and remains. • State jurisdictions may interpret Standards differently. State legislation will reflect this and will need to be taken into consideration before assessing this risk. • There was no research to demonstrate whether egg stamping reduced the incidence of human salmonellosis.	FSANZ 2.2.2 AECL Egg Stamping booklet Principles for Traceability / Product Tracing as a Tool Within a Food Inspection and Certification System (Codex CAC/GL 60-2006)
Contaminated egg stamping equipment	Cage / Barn / Free range	Should be cleaned on a regular basis	• The egg stamping equipment should be maintained in a clean state. • If inadequate grading processes are in place the possibility remains that cracked / broken / dirty eggs will be present at the terminal end of processing for stamping, which may contaminate the equipment, which can then cross-contaminate clean eggs (where the stamp actually contacts the egg). • Dirty egg stamping equipment can also result in illegible stamps on	AECL Egg Stamping booklet

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			the eggs. • An appropriate cleaning schedule may include daily visual assessment and weekly cleaning.	
Compressed air cleaning	Cage / Barn / Free range	A high level of care needs to be applied when using compressed air to clean equipment	• Grading equipment is often not designed in a manner that enables easy cleaning. • Therefore compressed air can be used to remove debris from hard to clean areas, however, this can aerosol any *Salmonella* present in the debris (dust, egg internal contents, dirt, faeces, etc.) around the grading area. • Infection can be obtained through *Salmonella* aerosols. • A high level of care is required when using compressed air for cleaning to ensure that the debris that is removed can be contained, and easily cleaned / removed from the grading area. • Compressed air may be appropriate to be used as part of line clearance before equipment cleaning. • There is considerable information relating to the risk of cross-contamination and spread of bacteria through the use of compressed air cleaning in the medical field, but limited in an egg grading environment.	104, 166

2.4 Pasteurised Egg (egg pulp) / Egg Products

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Definition of an egg processor	Egg Processors / Food Service	FSANZ Standard 4.2.5	• FSANZ Standard 4.2.5 defines an egg processor as: "*a business, enterprise or activity that involves:* *(a) pulping, separating, grading, packing, washing, candling, assessing for cracks or oiling eggs received from an egg producer; or* *(b) storing or transporting eggs in association with any of the activities in paragraph (a); or* *(c) processing egg product under clause 21 of this Standard*." • Clause 21 relates to pasteurisation (or its equivalent) required for egg pulp (defined as "*the contents of an egg, without sugar or salt*"), as unpasteurised egg pulp cannot be used for human consumption, as Standard 4.2.5 indicates that egg producers "*must not sell or supply eggs or egg pulp for human consumption if it knows, ought to reasonably know or to reasonably suspect, that the eggs are unacceptable*", and the definition of an unacceptable egg includes *"(b) egg product which has not been processed in accordance with clause 21*." • Standard 4.2.5 also indicates that "*This Standard does not apply to retail sale or catering activities*", which would exempt food businesses, although Standard 2.2.2 includes in the definition of an unacceptable egg "*(c) egg product which contains a pathogenic micro-organism, whether or not the egg product has been processed in accordance with clause 21.*" • There is considerable evidence that retail food businesses	79 FSANZ 4.2.5 FSANZ 2.2.2 Recommendation: Review the requirements of food service outlets to prepare raw egg products without using a raw egg product that has been processed through a verifiable process that reduces bacterial load.

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			routinely crack, separate and pulp eggs to produce raw egg products, which are of high food safety concern, with no verification that the process undertaken to produce the raw egg food minimised the bacterial growth / load. • Therefore, clarification is required as to how many eggs are processed before a business is classed as an egg processor and therefore must abide by requirements in clause 21.	
Other FSANZ Standards	Egg Processors / Food Service	Standards in Chapter 3 – Food Safety Standards	• The grading and packaging of eggs for human consumption is exempt from the FSANZ standards 3.2.2 and 3.2.3. • Standard 3.2.2 - Food Safety Practices and General Requirements and *3.2.3* - Food Premises and Equipment "*apply to processing under clause 21 and storage and transport under clause 22, but not to any other processing activities.*" • Therefore these standards only apply to pasteurisation processing. However, some state legislation has reversed this exemption.	FSANZ Chapter 3
Eggs to be pasteurised	Egg Processors / Food Service	Cracked and dirty eggs can be sent for pasteurisation	• FSANZ Standard 4.2.5 indicates that cracked and dirty eggs can be sent for pasteurisation to a licensed and approved egg processor. • An 'unacceptable egg' is also defined as "*egg product which contains a pathogenic micro-organism, whether or not the egg product has been processed in accordance with clause 21 (pasteurised).*" For example, if a batch of pasteurised egg product tests positive for *Salmonella,* it would be acceptable to re-process that batch until the	56, 104, 141, 222, 238 FSANZ 4.2.5 FSANZ 1.6.1 FSANZ Schedule 27

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			required testing produced the required results (FSANZ Standard 1.6.1 (Schedule 27)). **Note: State jurisdictions may interpret the Standards differently. State legislation will reflect this and will need to be taken into consideration.** • Pasteurised in shell eggs are eggs that are still in their shells (i.e. not cracked) and have been subjected to a process that significantly reduces bacterial contamination on the eggshell and to the centre of the yolk. • National Pasteurised Eggs (www.safeeggs.com) is a USA company that produces these eggs, which is looking to expand into Australia. • Time and temperature abuse of contaminated eggs before pasteurisation was shown to increase the risk of consumer illness from pasteurised product contaminated with *Salmonella* Enteritidis. • Pasteurisation is not a sterilisation process and the efficacy of the pasteurisation process will depend on the initial microbiological load of the eggs / pulp, therefore eggs to be sent for pasteurisation should be stored well below 7°C to minimise *Salmonella* growth. • There are suggestions that food service businesses may attempt to pasteurise eggs / egg pulp as a measure to reduce *Salmonella,* however, any pasteurisation process must be verified as effective for the reduction of *Salmonella*. • Eggs should be processed as soon as possible after they have been graded for pulping to minimise the time that bacteria can grow and therefore reduce the bacterial load	

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			to be reduced through pasteurisation.	
Insects / rodents / animals	Egg Processors	Minimise insect and rodent load near pasteurisation area; eggs to be pasteurised Have an adequate and regularly monitored rodent control program in place Replace baits as required.	• Insects (e.g. flies, beetles and cockroaches), rodents (including pet rodents) and animals such as chickens, turkeys, dogs, cats, pigs, cows, sheep, wild birds (e.g. ducks, pigeons, finches and sparrows) and other wild animals (such as foxes) are carriers for *Salmonella.* • Insects / rodents / animals can contaminate area and equipment, which can then cross-contaminate eggs for pasteurisation or egg pulp (before and after processing). Hence, there must be no insects / rodents / animals in a pasteurisation area. • Rodent bait stations must be monitored and re-baited regularly.	58, 80, 83, 94, 104, 123, 145, 151, 165, 188, 219
Egg cracking	Egg Processors	Eggs should be cracked at a reasonable distance from the pasteurisation plant / equipment, transported below 7°C if pasteurisation plant is not on the site of the grading area, and treated within 48 hours of eggs cracked	• Bacteria present on the eggshell poses the highest risk for contamination of egg pulp post-processing. • To reduce the risk of the pasteurised product and processing equipment becoming contaminated, the equipment should be kept at a reasonable distance from the finished pulp, and people handling eggshells should have limited access to the finished pulp. • Eggs that are to be cracked should be stored well below 7°C to limit *Salmonella* growth. • Eggs should be processed as soon as possible after they have been graded for pulping to minimise the time that bacteria can grow, and therefore reduce the bacterial load that is to be reduced through pasteurisation.	62, 91, 104, 141, 222, 238

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			• Anecdotally, it is easy to find Salmonella in raw egg pulp that has been sent for pasteurisation in Australia and there is evidence from non-Australian research to support this.	
Pasteurisation staff entering grading area / farm	Egg Processors	Grading area staff should not access pasteurisation area, and pasteurisation staff should not access the grading area (or farm) in the 24 hours prior to performing pasteurisation	• Staff performing pasteurisation should not be present in the grading area, or farm, in the 24 hours prior to pasteurisation. • Humans can carry *Salmonella* on clothes, shoes, skin, hair, etc., and in their gut, which can be transferred to eggs / egg products if appropriate interventions are not in place (such as hand washing facilities). • Egg yolks are an ideal growth medium for *Salmonella* and can easily be re-contaminated after pasteurisation. • *Salmonella* can grow to levels that can cause human illness within 2 hours (if stored at room temperature, and depending on initial bacterial load). • Maximum growth rate in yolk alone is significantly greater than whole eggs at all storage temperatures that have been investigated.	17, 74, 104, 172, 195
Ineffective pasteurisation process	Egg Processors / Food Service	Pasteurisation process should be verified, and any product must comply with FSANZ microbiological standards	• Pasteurisation is not a sterilisation process, therefore pasteurised product may be contaminated with bacteria. • Pasteurised egg product in Australia is only required to be tested for *Salmonella.* • FSANZ has a set of pasteurisation parameters, which will help produce a safe product. Any parameter that falls outside (below) of these parameters will result in an	56, 62, 91, 222 FSANZ 4.2.5 FSANZ 1.6.1

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			ineffective *Salmonella* reduction. • Ineffective pasteurisation of egg yolk presents the highest risk for human illness from contaminated / ineffectively processed pasteurised product. • Effective pasteurisation is more important for eggs / egg pulps that are sent for pasteurisation, as the *Salmonella* load can be higher than graded eggs.	
Dried egg product	Egg Processors / Food Service	Should be prepared using a verified process	• There are a number of recalls in countries other than Australia due to the presence of *Salmonella* in dried / powdered egg product. • *Salmonella* can persist in very dry (desiccated) environments, even after being subjected to very high temperatures. • Drying (desiccation) can increase some *Salmonella* resistance to various stressors, which will enable it to be revived easily. • *Salmonella* can form a biofilm (a biofilm is a bacterial protective 'coat' when it is in unfavourable conditions). • The rate of biofilm formation is inversely linked to the rate of desiccation.	9, 26, 85, 104

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Contamination of pasteurised egg pulp	Egg Processors / Food Service	Open containers of pasteurised egg product should be discarded after 24 hours if not stored below 7°C, and discarded within 3 days of opening	• Egg pulp is an ideal growth medium for many bacteria, including *Salmonella.* • Pasteurised egg pulp can be contaminated when exposed to air by environmental bacteria (such as present in dust, etc.). This is similar to pasteurised milk 'going off'. • Pasteurisation is not a sterilisation process so there may be bacteria still present in the pasteurised product. • Pasteurised egg product should be treated like a raw egg product once opened. It should be discarded: i) after 24 hours if not stored below 7°C, and ii) within 3 days of opening (as it is assumed that the product spent time out of required storage temperatures through access during service), and iii) subject to the 2hr / 4hr rule (put in the fridge within 2 hours, discarded after 4 hours at room temperature). • Inadequate pasteurisation temperatures and temperature abuse during post-pasteurisation storage were associated with increased risk of human illness with *Salmonella* Enteritidis.	104, 142, 222, 238

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Whole shell eggs and pasteurised eggs used in a single food product	Food Service	Pasteurised product should be used before the whole shell eggs, and both should not be present in the food preparation area at the same time	• Pasteurised eggs and whole shell eggs should not be present on the food preparation area at the same time. • Pasteurised eggs can easily become contaminated through cross-contamination from the shell of the whole eggs, especially if the pasteurised product isn't to be used in the preparation of the food. • Pasteurised product should be used first before handling whole shell eggs to minimise the chances of the pasteurised product that is to be re-stored becoming contaminated. • Food preparation staff must wash their hands after handling shell eggs. • Washing hands is very important if the food being prepared is raw or low-cooked egg product.	104, 142, 222, 238

2.5 Egg Storage and Transport

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Presence of water / moisture on eggs	Egg Producers / Wholesale / Food Service	Eggs must be transported and stored dry	• Eggs must not get wet at any stage through the transport and storage process. • *Salmonella* grows faster in the presence of water than in a dry environment and water also facilitates the movement of *Salmonella* into the egg. • The quality of the eggshell and the cuticle are significant determining factors for the rate of penetration of *Salmonella* inside the egg. • Egg washing may damage the cuticle so washed eggs are at a higher risk of bacterial penetration if water is present than is the case with unwashed eggs. • Cooling systems should not allow eggs, or equipment that eggs come into contact with, to become wet or produce condensation (referred to as 'sweating'). • Diffusion into the egg can occur due to differences in density/pressure and when the external environmental temperature is greater than the internal temperature of the egg. • Any external application to the egg must be at a temperature higher than the internal temperature of the egg (including eggshell) so that diffusion is from inside the egg to outside. • Research into *Salmonella* penetration into eggs has found that it is easier to artificially contaminate eggs with a wet solution of bacteria containing *Salmonella* as opposed to a	16, 53, 77, 104, 148, 189, 193, 241

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			dry faecal sample. • Eggs are not sterile and there is a possibility that *Salmonella* and or other pathogens will be present on eggshells. • To limit condensation on the eggs, they should be maintained in cold-chain once they have reached the required storage temperature.	
Presence of yolk if broken during transport / storage	Egg Producers / Wholesale / Food Service	Reduce the presence of yolk, particularly on equipment that has regular / sustained contact with eggs If yolk is present, clean and disinfect	• *Salmonella* grows rapidly in egg yolk as it is an excellent growth medium. • Bacterial growth rate in yolk alone is significantly greater than whole eggs at all storage temperatures that have been investigated. • 10^8-10^9 CFU/mL of *Salmonella* was achieved three days at 15°C, 26 h at 22°C, and 9 to 10 h at 37°C. • The dose of *Salmonella* required to cause human illness can be below 10^3 CFU. • Egg yolk should not be present on equipment that is in regular contact with eggs such as trays, etc. • Antibodies present in the yolk have been shown to have little effect on the growth of some types of *Salmonella*, but other studies have indicated there may be some inhibitory effect. • The efficacy of antibodies may be affected by a number of variables.	17, 31, 73, 74, 104, 141, 195, 220

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Insects / rodents / animals	Egg Producers / Wholesale / Food Service	Minimise insect / rodent / animal load where eggs are stored and during transport Have an adequate and regularly monitored rodent control program in place Replace baits as required.	• Insects (including flies, beetles and cockroaches), rodents (including pet rodents), livestock and pets including chickens, turkeys, dogs, cats, pigs, cows, sheep, wild birds (including ducks, pigeons, finches and sparrows) and other wild animals (such as foxes) are natural carriers for various types of *Salmonella*. • Insects / rodents / animals can contaminate any storage area and transport equipment, which can then cross-contaminate eggs. These insects / rodents / animals should not be present where eggs are stored and during transport. • Ensure rodent bait stations are monitored and re-baited regularly.	58, 80, 83, 94, 104, 123, 145, 151, 165, 188, 219
Eggs stored in a cool room before grading and/or washing	Egg Producers	Assess how long it takes for the internal egg temperature to reach the storage temperature Washed within 4 days (96 hours) of lay	• Unwashed eggs that are stored before washing should not be held for longer than 4 days. • Unwashed eggs, both dirty and clean-looking, will harbour bacteria on the eggshell, including *Salmonella*. • *Salmonella* will grow above 7°C, so the longer the eggs take to be graded the more likely the bacterial load will increase. • The longer *Salmonella* is in contact with an eggshell the more likely *Salmonella* will penetrate the egg, therefore unwashed eggs should be stored well below 15°C as soon as possible after collection. • Assess how long it takes for the internal temperature of the eggs to reach storage temperature as this could impact the growth of bacteria present inside the egg (as a result of diffusion at any stage of the laying process).	53, 104, 141, 148, 149, 158 AECL Code of practice for shell egg, production, grading, packing and distribution

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			• Need to consider if the storage conditions will result in condensation on the eggs when they are removed from storage for washing, as condensation on the eggs significantly increases the risk of bacterial penetration into the eggs and can allow bacteria to grow. • The 96 hours mentioned in the AECL code of practice could not be determined, however the recommendation would be to ensure that eggs are processed / graded as soon as possible to limit bacterial growth (including *Salmonella*).	
Inadequate airflow in cool room storage	Egg Producers / Wholesale / Food Service	Ensure adequate airflow is possible around the pallets of eggs	• Ensure airflow around the entire cool room is adequate to reduce the temperature of all eggs to the cool room temperature as fast as possible. This could be achieved through the use of fan units, for example. • Over-stacking the cool room will reduce the efficacy in of the cooling and eggs (particularly in the centre of the pallet) will not reach storage temperature (it is possible that some eggs may never reach storage temperature). • Allow space between pallets / stacks of eggs in the cool room. • Assess how long it takes for eggs to reach the cool room temperature, especially for unwashed eggs that contain dirt / faeces, etc. on the eggshell as *Salmonella*, will continue to grow until temperatures reach below 7°C. • Graded and ungraded eggs must be clearly separated if they are not able to be stored in a separate cool room (which is ideal to prevent the airflow becoming contaminated and contaminating the washed eggs).	104, 141, 148, 158

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			• No research could be found that investigates the efficacy of airflow to cool eggs but this is possibly due to the incredible number of variables that would impact on the cooling efficacy of the cool room (outdoor temperature / humidity, size of cool room, size / type of cooling unit, number of eggs, etc.).	
Ineffective stock rotation	Egg Producers / Wholesale / Food Service	The oldest eggs should be processed / sold first	• Eggs stored for any length of time anywhere through-chain should be rotated to ensure the oldest eggs are processed and/or sold first. • The longer *Salmonella* is present on the eggs the greater the risk, as Salmonella can diffuse into or be able to move into the egg internal contents. • The Yolk Mean Time (YMT) is how long it takes for the yolk membrane to breakdown and if *Salmonella* is present in the internal contents, then once the YMT is reached *Salmonella* may be able to enter the yolk easier. • Yolk is an ideal growth medium for *Salmonella*. • Research indicates *Salmonella* presence on eggshells declines more rapidly under refrigerated storage conditions (i.e. 4°C) with no persistence past 4 weeks, and storage at room temperature increased the persistence of *Salmonella* on eggshells (although total bacterial count was reported to either remain constant or increase). • No *Salmonella* persisted past 4 weeks on eggs at any storage conditions. This indicates that older eggs may be a reduced risk for *Salmonella* on the eggshell. However, the antibacterial properties in the egg degrade over time, which means older eggs are at greater risk of *Salmonella* growth in the internal contents if processing and/or storage	77, 104, 128, 141, 148, 171, 173, 184, 222, 241 AECL Code of practice for shell egg, production, grading, packing and distribution

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			conditions have enabled *Salmonella* to penetrate the eggshell. There are many variables that impact on *Salmonella* penetration into the eggs including how long *Salmonella* is present on the eggshell. • *Salmonella* persists better at lower temperatures in the internal contents of eggs, however, growth is minimal (or zero), which is a better outcome for *Salmonella* risk management than *Salmonella* growth (measurable growth occurs in less than an hour above 15°C). • Fluctuations in storage temperature have been shown to significantly increase the growth of *Salmonella.* • The presence of extraneous matter (e.g. faeces, feathers, dirt, etc.) on the eggshell significantly increased *Salmonella* persistence and eggshell penetration. • It is highly recommended that all eggs, old stock or new, unwashed or washed, be stored at below 7°C as this inhibits *Salmonella* growth. • Eggs that are stored above 15°C should be sold as soon as possible.	
Unclean / ineffective cool room / cooler unit	Egg Producers / Wholesale / Food Service	Bi-yearly assessment, cleaning and sanitisation	• The cool room should be cleaned and sanitised at least twice per year. • Ensure the cool room is dry before eggs are stored there again. • Care needs to be taken when cleaning areas where dirty eggs have been regularly stored, to ensure that they are cleaned appropriately to prevent bacterial build-up and possible contamination of clean eggs.	104, 158, 166

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			• The cool room fan units should be assessed twice per year for functionality, and should be cleaned regularly to remove debris and dust build-up. • Regular cleaning of the fan units will reduce the risk of *Salmonella* being continually cycled around the cool room if the unit becomes contaminated with *Salmonella* (this is possible if the air intake is from an area that may be contaminated with *Salmonella*). • Ensure that the water from the cooler unit does not pool or splash near eggs.	
Ineffective cool room temperature and humidity control	Egg Producers / Wholesale / Food Service	Ensure temperature / humidity is controlled in the storage area so that environmental fluctuations during seasons do not impact on the egg quality or bacterial growth.	• The temperature and humidity of the egg storage environment is a determinant of egg quality, not necessarily egg food safety. • The humidity and temperature should be balanced to ensure that the eggs do not 'sweat' (i.e. condensation forms on the outside of the egg), and bacterial growth is minimised. • *Salmonella* can grow below 15°C, but most *Salmonella* will stop growing at below 7°C (may not destroy bacteria but will limit growth). • Storing eggs at below 15°C (or even at below 7°C) may not always be appropriate to manage the risk of condensation forming on the eggs when they are removed from storage (e.g. in high humidity environments). • Condensation has been shown to assist *Salmonella* penetration into the egg. • A humidity of approximately 60-70% is considered	53, 104, 148, 173

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			appropriate for most environments to prevent condensation and limit bacterial growth, although it will still depend on environmental factors including the outside humidity. • The temperature and humidity should be monitored regularly, including measurement with external monitors as the inbuilt monitors may de-calibrate over time and lose accuracy.	
Inappropriate transport equipment / vehicle for whole eggs	Egg Producers / Wholesale / Food Service	Regularly cleaned Temperature below 7°C Do not damage or crack the eggs	• Any vehicle, and equipment within the vehicle, that is used to transport eggs should be cleaned regularly (at least once per week) to prevent the build-up of *Salmonella*. • *Salmonella* can be present on eggshells even after washing, as washing can reduce the bacterial load but it does not totally remove it (i.e. eggs are not sterile). • Any eggs that need to be transported for grading, processing, warehouse (including retailer) storage prior to the place of consumption, should be transported at a temperature below 7°C as *Salmonella* can grow at temperatures above this. • FSANZ Standard 4.2.5 clause 8 indicates that "*transportation vehicles are designed and constructed in a way that minimises the contamination of the eggs, allows for effective cleaning and sanitisation, and minimises the harbourage of pests and vermin; and keep premises, equipment and transportation vehicles effectively cleaned, sanitised and in good repair to ensure the eggs are not made unsafe or unsuitable."* • Transport vehicles and equipment used to transport eggs should be appropriate for the purpose and limit the incidence of eggs cracking through compounding	141, 173, 195, 241 FSANZ 4.2.5

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			mechanical stressors.	
Storage temperature of washed eggs	Egg Producers / Wholesale / Food Service	Washed eggs should be stored below 7°C	• Egg washing significantly increases the penetration of *Salmonella* into the eggshell as it damages the protective cuticle, which has antibacterial properties. • Washing and sanitising puts the eggs into contact with water, which significantly increases the risks of cross-contamination of clean eggs. This is especially when wash water is recycled. This can cause bacterial diffusion into the egg and facilitating the movement of motile bacteria such as *Salmonella*. • Egg washing must be done with extreme care and understanding of the risks. • Egg washing is not required in Australia but the sale of dirty eggs is prohibited under FSANZ Standard 2.2.2. • Eggs that have been washed must be stored below 7°C to reduce the ability of *Salmonella* to grow, so even if *Salmonella* was present on the surface or able to penetrate into the internal contents, the number of organisms would pose less risk of human illness than if storage conditions allowed *Salmonella* growth. • Research indicates that *Salmonella* presence on eggshells declines more rapidly under refrigerated storage conditions (i.e. 4°C) with no persistence past 4 weeks, and that storage at room temperature increases the persistence of *Salmonella* on eggshells (although total bacterial count was reported to either remain constant or increase). However, no *Salmonella* persisted past 4 weeks on eggs at any storage conditions.	19, 36, 38, 64, 67, 71, 72, 77, 128, 138, 171, 177, 184, 186, 195, 222, 240 FSANZ 2.2.2 Recommendation: Amend the AECL egg code of practice to indicate that all shell eggs for human consumption must be stored below 7°C through-chain upon leaving the grading area.

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			• This indicates that older eggs may be a reduced risk for *Salmonella* on the eggshell, however, the antibacterial properties in the egg degrade over time, which means that older eggs are at greater risk of *Salmonella* growth in the internal contents if processing and/or storage conditions have enabled *Salmonella* to penetrate the eggshell. There are many variables that impact on *Salmonella* penetration into the eggs including how long *Salmonella* is present on the eggshell. • *Salmonella* persists better at lower temperatures in the internal contents of the eggs, however, growth is minimal (or zero), which is a better outcome for *Salmonella* risk management than *Salmonella* growth (measurable growth occurs in less than an hour above 15°C). • Fluctuations in storage temperature have been shown to significantly increase the growth of *Salmonella.* • The presence of extraneous matter (e.g. faeces, feathers, dirt) on the eggshell significantly increased *Salmonella* persistence and eggshell penetration. • A WHO risk assessment of controlling *Salmonella* Enteritidis showed that keeping retail storage temperature at no more than 7.7°C reduced risk of illness per serving by about 60%. • In 1991, the USA amended its regulations to state that shell eggs for consumption must be at temperatures of no greater than 7.2°C upon leaving the grading area, and in 2000 published its final ruling that all retail establishments and other sales outlets for eggs, and businesses that use eggs to produce food for immuno-compromised individuals also had to abide by this rule.	

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			• A risk assessment of the US FDA rule found "*The predicted risk of salmonellosis from the consumption of eggs held and transported at 18.3°C and subsequently diverted to human consumption is 25 times higher than the risk when eggs are held and transported at 7.2°C.*" • There is considerable evidence to support storage of shell eggs below 7.2°C as a highly effective measure to reduce the risk of human salmonellosis compared to other interventions. • Although the WHO and USA measures, recommendations and assessments are based on controlling *Salmonella* Enteritidis, they would also be pertinent for *Salmonella* Typhimurium given that it has been reported to be able to grow below 7.2°C. • A risk assessment of the Australian egg industry and control of *Salmonella* Typhimurium found similar conclusions to WHO and US FDA assessments "*Refrigeration of eggs after processing and during wholesale and retail storage could substantially reduce the risk of human Salmonellosis. This option exerts the greatest impact on risk reduction compared to other strategies*." This measure would also help protect the Australian egg industry from catastrophic outcomes from a *Salmonella* Enteritidis outbreak in the industry.	
Storage temperature of eggs for pasteurisation	Egg Producers / Egg Processors / Wholesale	Eggs to be sent for pasteurisation should be stored below 7°C	• It may take some time for eggs to be sent for pasteurisation if the process is performed offsite and, if not stored properly, bacteria will be able to proliferate and damage the integrity of the eggs. • The pasteurisation process does not kill all bacteria	104, 141, 148, 149, 157, 222, 238 FSANZ 1.6.1

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			present (it is not a sterilisation process) so it is important that a low load of bacteria goes into the process, so that any reduction through pasteurisation is meaningful in terms of the safety of the final product and the ability of the product to meet microbiological limits under FSANZ Standard 1.6.1. • Risk analysis has found that reducing time and temperature abuse of contaminated eggs before pasteurisation reduced consumer risk of illness. • A risk assessment of the Australian egg industry found that "*Risk reduction could be brought about by reducing the storage time for eggs destined for pasteurisation.*"	
Raw egg pulp transport	Egg Producers / Egg Processors / Wholesale	Transported at a temperature below 5°C if chilled, and below -18°C if frozen.	• *Salmonella* can grow at temperatures below 15°C, however, growth is significantly suppressed at temperatures below 7°C. • Egg pulp can be contaminated with bacteria transferred from eggshells during the breaking / cracking process, which may include *Salmonella*. • Pasteurisation does not destroy all bacteria so the integrity of the final pasteurised product will depend on the bacterial load of the pulp to be processed. This is a requirement under FSANZ Standard 4.2.5. • Ensure any vessels used to transport egg pulp are cleaned and sanitised regularly, including the piping, connectors and valves used for filling and discharge of egg pulp. • FSANZ Standard 4.2.5 clause 19 indicates that "*transportation vehicles are designed and constructed in a way that minimises the contamination of the eggs or egg*	90, 104, 241 FSANZ 4.2.5 .

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			products, allows for effective cleaning and sanitisation, and minimises the harbourage of pests and vermin; and keep premises, equipment and transportation vehicles effectively cleaned, sanitised and in good repair to ensure the eggs or egg products are not made unsafe or unsuitable."	
Storage after pasteurisation	Egg Processors / Wholesale / Food Service	Stored below 7°C, and if frozen approximately -18°C	• Bacteria can still be present in pasteurised egg pulp after pasteurisation (depending on the time and temperature variables). Refrigeration below 7°C after processing will limit *Salmonella* growth. • Freezing does not destroy *Salmonella* but may reduce the load. It has been demonstrated that some foods protect *Salmonella* through freezing better than others, including liquid egg, out to periods of years. • FSANZ Standard 4.2.5 (clause 22) requires that "*A processor must ensure that egg product processed under clause 21 (i.e. pasteurisation or equivalent) is stored or transported under time and temperature conditions that control the growth of pathogenic micro-organisms.*" • Under clause 21, the storage conditions must be such that growth is 'controlled' (interpretation of 'controlled' could be subjective). • The final processing step outlined under FSANZ Standard 4.2.5 clause 21 indicates that pasteurised product must be rapidly cooled to ≤7°C. • Inadequate pasteurisation, and temperature abuse during post-pasteurisation storage were associated with increased risk of consumer illness with *Salmonella*	13, 71, 104, 138, 222, 238, 240

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			Enteritidis.	
Storage of dried / powdered egg product	Egg Processors / Wholesale / Food Service	As per manufacturer's instructions	• The storage conditions will depend on assessment by the manufacturer and/or egg processor as to the quality of the drying process to remove viable bacteria, including *Salmonella*. • *Salmonella* can survive in desiccated environments and the drying process may not remove all *Salmonella*. • Powdered egg has been recalled due to *Salmonella* contamination concerns, but it is unknown whether contamination occurred pre- or post-processing. • Powdered egg should be stored in a dry cool area until reconstituted, where it should be kept under refrigeration conditions and be treated as liquid pasteurised pulp, as *Salmonella* can grow in reconstituted powdered egg product.	9, 104
End user storage of eggs / eggs stored at room temperature during food service / egg product preparation	Wholesale / Food Service	Store eggs at <4°C after end-user purchase until required for use	• End users should store eggs below 4°C, especially if the eggs are to be used in raw or low-cooked egg products. • Storage below 4°C will not only maintain the quality of the egg but will also limit *Salmonella* growth, and reduce the ability of *Salmonella* to penetrate the egg (but not necessarily prevent penetration). • Most types of *Salmonella* can grow below 10°C, while most will not grow below 7°C, and bacterial load will actually decline with time (however, not all *Salmonella* will decline over time at these temperatures). • A risk assessment model indicated that Australian	13, 67, 99, 104, 128, 141, 148, 177, 195, 204, 222, 236 Code of hygienic practice for precooked and cooked foods in mass catering (CAC/RCP 39-1993) UTAS Risk Ranger

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			variables (industry, climate, egg use, etc.) meant that risk is reduced if the end user stores eggs in the fridge (i.e. below 4°C). • Low storage temperatures and appropriate handling techniques including washing hands after handling whole eggs are critical to limit cross-contamination to other foods in the kitchen. • Eggs subjected to fluctuations in storage temperature were at a higher risk of *Salmonella* proliferation in a study investigating *Salmonella* Enteritidis but not on *Salmonella* Typhimurium. • Eggs should not be left out of storage for a time that allows the internal temperature to rise to a level that will permit the growth of *Salmonella* (above 7°C), and should not be placed in a position that is near a heat source. • High temperatures will cause issues to the quality of the egg as the internal contents degrade when subjected to fluctuations in temperature. • In cases where an egg product recipe requires eggs to be at room temperature before use, eggs should be used promptly and treated as any other perishable food item. The time it will take for the internal contents to reach room temperature will depend on a number of variables including the room temperature itself and the external and internal quality of the egg. • It is recommended that eggs that have been at room temperature for more than 2 hours be discarded (or if re-refrigerated, they not be used for raw or low-cooked egg products).	

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Storage temperature of chilled raw foods	Wholesale / Food Service	Store below 4°C and discarded after 24 hours	• Most foodborne illness outbreaks have involved temperature abuse of raw or low-cooked eggs, and AECL has prepared a report on the various uses of eggs to identify the types of dishes that may pose a greater food safety risk. • The rate of *Salmonella* contamination in Australia has been estimated to be <1 egg: 20,000, any situation that allows bacteria to proliferate will increase the risk of human illness from raw egg food. • Any raw egg foods (whether prepared with pasteurised egg or not) should be kept below 4°C and be discarded at the end of the food service day (no longer than 24 hours from time of manufacture) as the risk from cross-contamination from raw eggshells within a kitchen environment are high. • *Salmonella* Enteritidis grows significantly in the presence of eggs that have been subjected to fluctuations in temperature, indicating that raw egg foods should be stored and maintained under refrigerated conditions as soon as possible (e.g. constant retrieval of raw egg food from refrigerated conditions to room temperature and back should be discouraged). • Pasteurisation does not destroy all bacteria as it is not a sterilisation process, but the risks associated with producing raw egg products are reduced by using pasteurised eggs. • Any *Salmonella* present on whole shell eggs (or remaining in the pasteurised egg) can be transferred into the raw egg product and grow, especially if the food is held at temperatures between 10°C and 50°C (the rate of growth will also depend on additional variables).	17, 74, 79, 104, 141, 177, 204, 222, 237 AECL Culinary uses of eggs Australian Department of Health – Foodborne illness in Australia 2010 Code of hygienic practice for precooked and cooked foods in mass catering (CAC/RCP 39-1993) UTAS Risk Ranger Recommendation: Raw egg foods should be discarded at the end of the food service day, without exception.

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			• *Salmonella* grows most rapidly in egg yolk as it is an excellent growth medium, and the growth rate in yolk alone is greater than whole eggs at all storage temperatures that have been investigated. • 10^8-10^9 CFU/mL of *Salmonella* was achieved three days at 15°C, 26 hours at 22°C, and 9 to 10 hours at 37°C. • The dose of *Salmonella* required to cause human illness can be below 10^3 CFU. • Peer-reviewed articles that investigated similar effects in raw egg foods could not be found, however, a model for the prediction of *Salmonella* growth under varying temperature conditions has been developed. The University of Tasmania has developed a 'Risk Ranger' tool that "*is a simple food safety risk calculation tool intended as an aid to determining relative risks from different product, pathogen and processing combinations.*" • Raw foods made from pasteurised eggs should be discarded at the end of the food service (as it is assumed that the product spent time out of required storage temperatures through access during service), and subject to the 2hr / 4hr rule (put in the fridge within 2 hours, discarded after 4 hours at room temperature).	

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
Refrigeration of cooked food prepared with whole shell eggs	Wholesale / Food Service	Refrigerated immediately if not consumed immediately Discarded after 4 hours if left at temperature >4°C	• Cooking of egg products above 60°C (depending on time and temperature) will result in the destruction of most bacteria, including *Salmonella*. • Food prepared with whole fresh eggs should be refrigerated within 2 hours of preparation and discarded if held at room temperature for more than 4 hours. • Most *Salmonella* can grow above 10°C and can survive below 4°C (but should not grow). • Preservatives will impact on the ability of *Salmonella* to grow in the egg product, however, there is some evidence to suggest that *Salmonella* can become resistant to various preservatives (preservatives should be assessed as appropriate for purpose).	66, 104, 222, 237
Storage of frozen raw egg foods	Wholesale / Food Service	Raw egg foods for frozen storage should be placed at below -18°C as soon as possible	• Any raw or low-cooked egg products should be stored at -18°C as soon as possible after completion of the preparation, to limit the growth of *Salmonella.* • Freezing does not destroy *Salmonella*, but may reduce the load over time and it has been demonstrated that some foods protect *Salmonella* through freezing better than others, including liquid egg (out to periods of years, e.g. ice cream).	13, 104
Transportation temperature of pre-prepared egg products	Wholesale / Food Service	Transport below 5°C	• Egg products that have been pre-prepared and transported to their consumption destination (such as from a wholesaler or through a caterer) should be transported below 5°C, as there is a risk of bacterial proliferation if they are raw or low-cooked egg products, as there is no bacterial kill step in the food preparation.	177, 204 *Codex Code of Hygienic Practice for the Transport of Foods in Bulk and Semi-Packed Food*

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			• Off-site preparation of raw or low-cooked egg products carries a significant risk due to the additional time and possible temperature abuse that may arise due to transportation delays and serving constraints. • Epidemiological surveys have concluded that mass catering in general is the highest risk for human foodborne illness outbreaks. • If heated transportation is required, then the guidelines set out in Codex CAC/RCP 39-1993 should be followed, as fluctuations in temperature can cause significant increase in *Salmonella* Enteritidis growth rate in eggs / egg products. • Peer-reviewed articles that investigated similar effects in raw egg foods could not be found, but a model for the prediction of *Salmonella* growth under varying temperature conditions has been developed. The University of Tasmania has developed a 'Risk Ranger' tool that "*is a simple food safety risk calculation tool intended as an aid to determining relative risks from different product, pathogen and processing combinations*."	(CAC/RCP 47-2001 *Code of hygienic practice for precooked and cooked foods in mass catering* (CAC/RCP 39-1993) UTAS Risk Ranger
Cooling and reheating pre-prepared egg products	Wholesale / Food Service	Limit temperature abuse	• Epidemiological surveys have concluded that mass catering in general is the highest risk for human foodborne illness outbreaks. • The rate (and therefore load) of *Salmonella* in a reheated food will depend on the initial load of *Salmonella* present and the temperatures to which the food was cooled and then reheated. • Catered functions often do not have sufficient capacity to refrigerate or re-heat foods, hence there is significant risk	17, 74, 141, 177, 204 Code of hygienic practice for precooked and cooked foods in mass catering (CAC/RCP 39-1993) UTAS Risk Ranger

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			for human illness from off-site prepared egg products (particularly raw and low-cooked). • Codex CAC/RCP 39-1993 states that *Reheating must also be rapid so that the food passes quickly through the hazardous temperature range between 10°C and 60°C. This will usually require the use of forced air ovens, infrared or microwave reheaters. The temperature of the heated food should regularly be checked.*" • Consumers should be advised through signage or otherwise that the product contains raw eggs and attempts should be made to prepare raw egg products to order at the place of consumption. • There has been a significant increase in *Salmonella* Enteritidis growth rate and presence in eggs that have been subjected to fluctuations in temperature. • Adhere to the 2hr / 4hr rule (refrigerate before 2 hours, discard after 4 hours at room temperature). • *Salmonella* grows most rapidly in egg yolk as it is an excellent growth medium, and the growth rate in yolk alone is significantly greater than whole eggs at all storage temperatures that have been investigated. • 10^8-10^9 CFU/mL of *Salmonella* was achieved three days at 15°C, 26 hours at 22°C, and 9 to 10 hours at 37°C. • The dose of *Salmonella* required to cause human illness can be below 10^3 CFU. • Peer-reviewed articles that investigated similar effects in raw egg foods could not be found, however, a model for the prediction of *Salmonella* growth under varying	

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			temperature conditions has been developed. • The University of Tasmania has developed a 'Risk Ranger' tool that "*is a simple food safety risk calculation tool intended as an aid to determining relative risks from different product, pathogen and processing combinations.*"	
Hot-holding of egg foods	Wholesale / Food Service	Verified as appropriate for the food / process	• Egg products that are prepared as hot dishes should be stored in a hot-holding area as soon as possible to limit cooling of the product. • Raw or low-cooked egg products held in a hot-holding area (e.g. bain-marie) should not be held for more than 20 minutes before consumption (this includes as part of a sauce applied to a dish before hot-holding). • No basis could be found for the 20-minute recommendation, however, it could be assumed that bacterial growth would not be extensive in 20 minutes under most conditions. • The purpose of hot-holding is to ensure the food reaches the consumer at the required temperature and therefore may not be adequate for control of bacterial growth. • If the holding temperature is less than 45°C (which is why the recommendation is to hold no longer than 20 minutes), it may promote bacterial growth. • The temperature of the holding area should be validated using an external device to ensure the area is at the required temperature as the in-built temperature gauges can de-calibrate.	13, 104, 141, 222 Code of hygienic practice for precooked and cooked foods in mass catering (CAC/RCP 39-1993)

2.6 Egg and Egg Product Handling and Hygiene

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
FSANZ Standards Chapter 3	Food Service / Egg Producers / Wholesale / Egg Processors	Businesses that handle eggs and produces any type of egg product (raw, low-cooked or cooked) or egg food must understand their food safety requirements under state and federal legislation	• FSANZ Standards Chapter 3 applies to "*a business, enterprise or activity (other than primary food production) that involves –* *(a) the handling of food intended for sale; or* *(b) the sale of food; regardless of whether the business, enterprise or activity concerned is of a commercial, charitable or community nature or whether it involves the handling or sale of food on one occasion only*." • Some state jurisdictions may have made amendments to this during implementation, and may now require that primary food production must also adhere to standards within this Chapter. • These standards mirror requirements in the Codex General Principles of Food Hygiene, with considerations for Australian variables.	FSANZ Chapter 3 General Principles of Food Hygiene (Codex CAC/RCP 1-1969)
Staff food safety training	Food Service / Egg Processors	A working knowledge of the risks associated with different food items that contain eggs is essential	• Human error and/or the mishandling of food account for a significant proportion of *Salmonella* foodborne illness outbreaks linked to the consumption of egg products. • Any staff who are preparing high-risk foods such as raw or low-cooked egg products, must have a high level of understanding of the risks involved and the importance of proper preparation and food handling. • Appropriate practices should be identified and conveyed via training to staff who are preparing, handling or serving food such as raw or low-cooked egg products.	13, 79, 104, 222, 241 General Principles of Food Hygiene (Codex CAC/RCP 1-1969) AECL Culinary uses of eggs

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			• A foodborne illness outbreak is often devastating to a retail food business. It is important that all staff are aware of appropriate practice for handling products / foods that pose a higher food safety risk than others. • AECL has prepared a report on the various uses of eggs to identify the types of dishes that may pose a greater food safety risk.	
Inappropriate building / facility design	Food Service / Wholesale	Buildings / facilities where egg products are produced should be designed to allow adequate cleaning / sanitisation, and segment food preparation sections	• FSANZ Standards Chapter 3 and the Codex report (General Principles of Food Hygiene), outline what should be assessed to identify appropriate facility design and cleaning / sanitisation. • Facilities that produce egg products, particularly raw and low-cooked, should have regular cleaning / sanitisation procedures in place, and should consider designated 'clean' and 'dirty' areas for food preparation.	FSANZ Chapter 3 General Principles of Food Hygiene (Codex CAC/RCP 1-1969)
Personal health, hygiene and behaviour	Food Service / Egg Producers / Wholesale / Egg Processors	Any personnel who are sick should not handle eggs, or prepare or handle raw egg products / foods, including during the period up to 48 hours after recovery from symptoms	• Good personal hygiene and behaviour (no coughing, sneezing, gastrointestinal upset, regular hand washing and good personal hygiene, etc.) are required when handling whole egg / egg internal contents. • Human pathogens can potentially spread to other humans through the contamination of eggshells (which can contaminate other surfaces) or egg internal contents, which are ideal conditions for the growth of many bacteria / viruses / fungi. • Humans can also carry pathogenic *Salmonella* asymptomatically and can infect others through	12, 13, 18, 60, 82, 101,104, 132, 172, 206 FSANZ Chapter 3 General Principles of Food Hygiene (Codex CAC/RCP 1-1969)

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			contaminated food, or direct or indirect contact. • Research shows that both symptomatic and asymptomatic food handlers have caused (or are suspected to have caused) foodborne illness outbreaks. • The Australian Government Department of Health has produced a set of guidelines for managing food handlers during *Salmonella* outbreaks. • *Salmonella*-infected food handlers would be excluded from work until symptoms have ceased in Victoria and Tasmania, and/or for up to 48 hours after symptoms cease in the ACT, NSW, QLD, SA, NT and WA. **Note: Humans can still shed *Salmonella* for weeks or months after infection**.	
Sale of cracked and/or dirty eggs	Food Service / Egg Producers / Wholesale / Egg Processors	Any seller of eggs (primary production, wholesale, retail) must not sell cracked and/or dirty eggs	• FSANZ Standard 2.2.2 applies to any business that handles eggs. Selling visibly cracked and/or dirty eggs is prohibited by federal and state legislation. • To minimise cracking through-chain the compounding mechanical stressors must be well managed to ensure that eggs are not cracked at any stage (except in the preparation of egg products / food). • Every time eggs are subjected to mechanical stress (where some sort of force is applied to them) there is the risk that fractures can be created in the eggshell which can lead to cracks. • *Salmonella* can enter eggs more easily if cracks are present, however, it is unknown what size crack is required before it poses a risk, as some *Salmonella* strains can	27, 148, 195, 228 FSANZ 2.2.2

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			penetrate intact eggshells.	
Presence of yolk	Food Service / Egg Producers / Wholesale / Egg Processors	Reduce the presence of yolk, particularly on equipment, utensils and surfaces Clean and disinfect if yolk is present	• *Salmonella* growth rate in yolk alone is greater than whole eggs at all storage temperatures that have been investigated, as yolk is an excellent growth medium. • 10^8-10^9 CFU/mL of *Salmonella* was achieved three days at 15°C, 26 hours at 22°C, and 9-10 hours at 37°C. The dose of *Salmonella* required to cause human illness can be below 10^3 CFU. • Yolk must not be present on equipment, utensils and surfaces that come into contact with other equipment / utensils / surfaces and food. • Spilled yolk must be cleaned up promptly to prevent *Salmonella* growing to levels that increase the risk of cross-contamination. • Antibodies present in the yolk have little effect on the growth of some types of *Salmonella*, but other studies have indicated there may be some inhibitory effect. The efficacy of antibodies may be affected by a number of variables.	17, 31, 73, 74, 104, 141, 195, 220
Preparation of raw and low-cooked egg products	Food Service / Wholesale	Appropriate methods should be used to prepare raw and low-cooked egg products with low *Salmonella* growth potential	• In Australia, a significant proportion of foodborne illness outbreaks have involved temperature abuse of raw or low-cooked eggs products. • AECL has prepared a report on the various uses of eggs to identify the types of dishes that may pose a greater food safety risk. • The control of *Salmonella* in the kitchen environment is	11, 55, 87, 102, 130, 131, 146, 156, 167, 182, 183, 192, 212, 213, 243 AECL Culinary uses of eggs

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			complex. • If *Salmonella* is present in foods with low water activity (such as chocolate and peanut butter), it takes a longer time / temperature treatment to destroy the bacteria (up to a year at ambient temperature) than if the *Salmonella* is present in a food with high water activity (e.g. raw egg mix). • Sugar can aid in the heat resistance of *Salmonella* as it reduces the water activity of the food. • It can take longer to destroy *Salmonella* at pH 5.5 than at pH 8. Other variables such as rate of change will impact on the destruction rate and *Salmonella* resistance to environmental conditions. • There have been a number of outbreaks associated with highly acidified products (apple cider, orange juice). • If using pH to control *Salmonella* growth, it has been shown that a period of time (days) at ambient temperature is required BEFORE refrigeration of the acidified product to achieve *Salmonella*-free status. • Initial heat treatment with sub-lethal temperatures can increase the resistance of *Salmonella* through the stimulation of the bacterial expression of thermo-tolerant proteins. • The type of preparation method has been shown not to affect the destruction of *Salmonella,* which indicates that the important variables are time / temperature. • It is important that appropriate practices are identified and conveyed to staff who are preparing, handling or serving	

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			food, particularly raw or low-cooked egg products. • The implications of a foodborne illness outbreak in a retail food business are often devastating, so it is in the interests of the business owner to ensure that all staff are aware of appropriate practice for handling products / foods that pose a higher food safety risk.	
Cross-contamination of utensils, equipment, or surfaces with egg internal contents, eggshells or bacteria present on the eggshell	Food Service / Egg Producers / Wholesale / Egg Processors	Ensure all utensils, equipment or surfaces that come into contact with egg internal contents or eggshell are appropriately cleaned and sanitised (or equivalent) prior to coming into contact with other food	• Bacteria present on the eggshell can be transferred to any surface it comes into contact with and therefore can cross-contaminate food that comes into contact with that surface. • Bacteria and viruses are able to grow in raw egg products, so cross-contamination of bacteria from other raw ingredients into a raw egg product must be avoided. • Kitchen towels and sponges that are used to clean and/or dry hands, utensils, surfaces or equipment can harbour *Salmonella* and can cross-contaminate raw egg products. • The efficacy of hand washing is voided if hands are dried on a contaminated kitchen towel, as *Salmonella* can survive on kitchen towels that are allowed to dry without appropriate cleaning • Research that assesses commercial facilities found that, although overall the general hygiene practice was better than that in domestic kitchens, pathogenic bacteria were still detected. • Common disinfectants used to remove *Salmonella* are not always effective, especially when *Salmonella* had formed a biofilm (a biofilm is a bacterial protective 'coat', formed during unfavourable conditions). • Food containing raw egg should be prepared in a way that	24, 30, 41, 76, 139, 140, 143, 152, 173, 196, 197, 207, 221

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			prevents any cross-contamination, and it should be ensured that the food is consumed within 2 hours (if stored at ambient temperature), as there is often no bacterial kill step involved in the food preparation process. • A study involving *Salmonella* Enteritidis demonstrated that both dry and wet contaminated egg droplets were able to cross-contaminate other foods and surfaces. • The invasive capacity of *Salmonella* spp. depends on the environment (enrichment) available, therefore *Salmonella* present in an environment with substantial enrichment, like in food service where there are innumerable sources of enrichment (e.g. salt and sugars), is of much greater risk to human health than *Salmonella* present in an environment with limited enrichment. • The longer an egg product is at an ambient temperature and *Salmonella* is present, the higher the risk of illness due to the increased bacterial load and that potentially has an increased invasive capacity. • Using eggshells to separate eggs should be discouraged, particularly for the preparation of raw or low-cooked egg products, although no research could be found that explicitly assessed the transfer of bacteria from the eggshell during egg separation. • Food preparation equipment with multiple components is at high risk of cross-contamination due to the difficulty in cleaning them appropriately. • Stick mixers / blenders, should be taken apart, as water can get into the components during washing and, if not cleaned thoroughly, bacteria can proliferate and	

Inputs (Potential Hazard)	Applicable to	Minimum consideration to control risk	Why it is a risk, how to control it and other considerations	Additional Reading References
			contaminate any foods the equipment is used to prepare. • In Australia there have been a number of foodborne illness outbreaks whereby the probable source for cross-contamination may have the use of *Salmonella* contaminated stick mixers / blenders. The risk is especially high if the equipment is used subsequently to prepare other raw foods without appropriate cleaning.	
Raw or low-cooked egg products / foods served to vulnerable populations (e.g. immune-compromised individuals or elderly)	Food Service	Raw or low-cooked egg products should not be served to vulnerable populations unless all requirements under FSANZ Standard 3.3.1 have been met (if the food is intended for six or more vulnerable persons)	• Raw egg products / foods are at a higher risk of bacterial contamination as bacteria are able to grow in egg internal contents (contamination after cracking for food preparation), and bacteria that may be present on the eggshell can easily be transferred into the food during egg cracking and/or separation. • Vulnerable populations are more susceptible to foodborne illness and therefore the infective dose of bacteria that can make them ill is often low. • Vulnerable populations should not be served high-risk foods such as raw egg products, particularly products that have been prepared with unpasteurised egg pulp (i.e. with no bacterial kill step). • FSANZ Standard 3.3.1 specifically outlines food safety requirements for foods to be served to vulnerable populations (if the food is intended for six or more vulnerable persons). • There is a USA company (National Pasteurised Eggs) (www.safeeggs.com) that produces shell-pasteurised eggs, but these are not currently not available in Australia.	173, 222, 237 FSANZ 3.3.1

3 Recommendations

Summary of recommendations made throughout the risk identification tables:

- Review FSANZ standard 4.2.5 and consider removal of clause 9 or provide more clarity on producer responsibility.
- Update the AECL code of practice to indicate best before date as 6 weeks or less from estimated date of lay rather than date of pack.
- Update the AECL egg safety flyer as some of the advice requires adjustment (e.g. hands should be washed after handling ANY egg).
- Assess whether it is feasible to update FSANZ Standard 4.2.5 and 2.2.2 to prohibit wet floor eggs for human consumption as shell eggs (even after washing to remove dirt).
- Assess whether producers should be registered or licensed to wash eggs.
- Terminal sanitisers be allowed for use in Australian egg production to provide clarity to producers already undertaking this practice and to align with the USA. FSANZ to comment?
- Review the requirements of food service outlets to prepare raw egg products without using a raw egg product that has been processed through a verifiable process that reduces bacterial load.
- Amend the AECL egg code of practice to indicate that all shell eggs for human consumption must be stored below 7°C through-chain upon leaving the grading area. (update the AECL code of practice in general as many of the food safety references and FSANZ links are out of date).
- Raw egg foods should be discarded at the end of food service day, without exception.

4 References

1. Anon (2003). *Salmonella* in domestic animals. New York, NY, USA, CABI Publishing.

2. Anon (2012). "Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: annual report of the OzFoodNet network, 2010." Commun Dis Intell Q Rep **36**(3): E213-241. http://www.ncbi.nlm.nih.gov/pubmed/23186234
This report summarises the incidence of diseases potentially transmitted by food in Australia and details outbreaks associated with food in 2010. OzFoodNet sites reported 30,035 notifications of 9 diseases or conditions that are commonly transmitted by food. The most frequently notified infections were *Campylobacter* (16,968 notifications) and *Salmonella* (11,992 notifications). The most frequently notified *Salmonella* serotype was *Salmonella* Typhimurium, accounting for 44% of all *Salmonella* notifications. OzFoodNet sites also reported 1,640 outbreaks of gastrointestinal illness affecting 30,193 people and resulting in 722 people being hospitalised. There were 89 deaths associated with these outbreaks. The majority of outbreaks (81%, 1,330/1,640) were due to person-to-person spread, 9% (154/1,640) were suspected or confirmed to have been transmitted by contaminated food, 9% (155/1,640) had an unknown mode of transmission and 1 outbreak was due to transmission from animal to person. Foodborne and suspected foodborne outbreaks affected 2,146 persons and included 157 hospitalisations.Fifteen deaths were reported during these outbreaks. *Salmonella* was the most common aetiological agent identified in foodborne outbreaks and restaurants were the most frequently reported food preparation setting. A single food source was identified for 43 outbreaks, 21 of which were associated with the consumption of dishes containing raw or minimally cooked eggs; the majority (n=20) due to *S.* Typhimurium. These data assist agencies to document sources of foodborne disease, develop food safety policies, and prevent foodborne illness.

3. (FAO), F. a. A. O. (2003). "Egg Marketing - A guide for the production and sale of eggs." FAO Agricultural Services Bulletin **150**. http://www.fao.org/docrep/005/y4628e/y4628e00.htm
This guide provides information and advice to those concerned with the production and sale of eggs in developing countries with an emphasis on marketing, i.e. producing in order to meet market demand. Market-led egg production enables long-term business survival, higher profits and a better standard of living for the egg producer. Improvement measures discussed in this publication have been found to be effective in practice. However, as is inevitable with any publication attempting to address such a wide range of conditions, some of the recommendations and observations found herein may be unsuitable to the reader's particular circumstances. The reader should select what appears advantageous for the solution of his or her own particular problem(s).

4. Arnold, M. E., F. Martelli, et al. (2014). "Estimation of the rate of egg contamination from *Salmonella*-infected chickens." Zoonoses Public Health **61**(1): 18-27. http://www.ncbi.nlm.nih.gov/pubmed/23398774
Salmonella enterica serovar Enteritidis (*S.* Enteritidis) is one of the most prevalent causes for human gastroenteritis and is by far the predominant *Salmonella* serovar among human cases, followed by *Salmonella* Typhimurium. Contaminated eggs produced by infected laying hens are thought to be the main source of human infection with *S.* Enteritidis throughout the world. Although previous studies have looked at the proportion of infected eggs from infected flocks, there is still uncertainty over the rate at which infected birds produce contaminated eggs. The aim of this study was to estimate the rate at which infected birds produce contaminated egg shells and egg contents. Data were collected from two studies, consisting of 15 and 20 flocks, respectively. Faecal and environmental sampling and testing of ovaries/caeca from laying hens were carried out in parallel with
(i) for the first study, testing 300 individual eggs, contents and shells together and
(ii) for the second study, testing 4000 eggs in pools of six, with shells and contents tested separately. Bayesian methods were used to estimate the within-flock prevalence of infection

from the faecal and hen post-mortem data, and this was related to the proportion of positive eggs. Results indicated a linear relationship between the rate of contamination of egg contents and the prevalence of infected chickens, but a nonlinear (quadratic) relationship between infection prevalence and the rate of egg shell contamination, with egg shell contamination occurring at a much higher rate than that of egg contents. There was also a significant difference in the rate of egg contamination between serovars, with *S.* Enteritidis causing a higher rate of contamination of egg contents and a lower rate of contamination of egg shells compared to non-*S.* Enteritidis serovars. These results will be useful for risk assessments of human exposure to Salmonella-contaminated eggs.

5. Bain, M. M., I. C. Dunn, et al. (2006). "Probability of an egg cracking during packing can be predicted using a simple non-destructive acoustic test."
Br Poult Sci **47**(4): 462-469.
http://www.tandfonline.com/doi/full/10.1080/00071660600829233#abstract
1. The aim of this investigation was to test the predictive power of the dynamic stiffness measurement to identify eggs which are most likely to crack under field conditions.
2. A representative sample of eggs (n = 1660) was collected from the front of the cages in a commercial battery unit. Egg weight, % damping and dynamic stiffness (Kdyn) were recorded using an acoustic crack detection device. Intact eggs were marked and replaced in the front of the cages. These eggs were subsequently passed through online collection, grading and packing machinery, along with a volume of unmarked eggs. At the end of packing the acoustic test was repeated on the marked eggs, and these were subsequently categorised as being either intact (0) or cracked (1).
3. A logistic regression of the probability of cracking vs Kdyn revealed that as the Kdyn measurement decreases below 15,000 N/m there is a rapid increase in the probability that an egg will crack during routine handling.
4. Additional variables (visit, egg weight,% damping and position in the house (battery [1 to 7], side [1, 2] and tier [1 to 8]) were also fitted to the model but only egg weight, visit and tier effects significantly improved the model fit.
5. This study confirms that the dynamic stiffness measurement can predict the probability of an egg cracking in the field and with high precision. As this measurement also has a high heritability, it could be incorporated into breeding programmes, where it would offer an excellent method to improve eggshell quality and reduce the incidence of cracked eggs.

6. Barbezange, C., G. Ermel, et al. (2000). "Some safety aspects of *Salmonella* vaccines for poultry: in vivo study of the genetic stability of three *Salmonella* Typhimurium live vaccines."
FEMS Microbiol Lett **192**(1): 101-106.
http://www.ncbi.nlm.nih.gov/pubmed/11040436
Live vaccine strains of *Salmonella* should be avirulent, immunogenic and genetically stable. Some isolates of three commercially available live vaccine strains of *Salmonella* Typhimurium, sampled during a study on their persistence in a vaccinated flock of chickens, were analyzed for genetic stability using macrorestriction analysis of their genome. Two out of the three vaccine strains showed genetic instabilities. Two of the 51 isolates of Zoosaloral vaccine strain and nine of the 32 analyzed isolates of chi(3985), a genetically modified organism, were variants and showed different macrorestriction profiles.

7. Barbezange, C., F. Humbert, et al. (2000). "Some safety aspects of salmonella vaccines for poultry: distribution and persistence of three *Salmonella* Typhimurium live vaccines."
Avian Dis **44**(4): 968-976.
http://www.ncbi.nlm.nih.gov/pubmed/11195656
The purpose of this study was to analyze the safety characteristics of three commercially available live *Salmonella* vaccine strains (vacT, Zoosaloral, and X3985) in relation to their persistence in individual animals but also within a flock and in the environment. In a first experiment, the digestive and systemic distributions in chickens were followed for 10 days in individually reared chickens that were orally inoculated at 1 day of age. Strain X3985 quickly disappeared from the digestive tract but remained in the liver until the end of this experiment, whereas strains vacT and Zoosaloral colonized the liver as well as the gut for 10 days. In the second trial, behavior of the vaccine strains was studied in groups of 20 chickens during 10 wk after a single oral administration to individual birds. Strain vacT remained in the environment of inoculated animals for 4-5 wk. Six weeks after the inoculation, vacT was not

recovered from internal organs such as liver and spleen, and vacT disappeared from the digestive tract between the sixth and the 10th weeks. Comparatively, both Zoosaloral and X3985 vaccine strains persisted longer in the environment (8 wk at least). Of the vaccine strains, X3985 showed the greatest colonization of both systemic and digestive organs.

8. Barker, J., M. Naeeni, et al. (2003). "The effects of cleaning and disinfection in reducing *Salmonella* contamination in a laboratory model kitchen."
J Appl Microbiol **95**(6): 1351-1360.
http://www.ncbi.nlm.nih.gov/pubmed/14633010
AIMS: To establish a laboratory model to compare the effectiveness of detergent-based disinfection procedures for reducing cross-contamination risks during handling of contaminated chicken.
METHODS AND RESULTS: During handling of chickens, artificially contaminated with *Salmonella* enteritidis PT4, the organism was widely spread to hands, cloths, and hand- and food-contact surfaces. Hygiene procedures were assessed on the basis of their ability to reduce the number of recoverable salmonellas to <1 CFU. Although detergent-based cleaning using a typical bowl-wash routine without rinsing produced some risk reduction (from 100 to 61.4% of contaminated surfaces), it was insufficient to consistently restore surfaces to a hygienic state. By combining detergent-based cleaning with a rinsing step or with hypochlorite at 500 ppm (of available chlorine) some further reduction in microbial risk was achieved, but was not considered satisfactory for food hygiene purposes. By contrast the risk reduction produced by hypochlorite at 5000 ppm was highly significant and was sufficient to reduce the number of contaminated surfaces to 2.9%.
CONCLUSIONS: A key step in achieving a hygienic state through detergent-based cleaning is rinsing but even this will not produce a 'hygienic' result for difficult surfaces such as the chopping board or the dishcloth. Disinfectant compounds should be considered in order to reduce the potential for foodborne cross infection within the home environment.
SIGNIFICANCE AND IMPACT OF THE STUDY: Although tests are available to determine the performance of disinfectants, there are no quantitative procedures available to compare the risk reduction achieved by disinfection with that produced by detergent-based procedures. This study describes a reproducible laboratory method which can be used to differentiate the effectiveness of different hygiene procedures for reducing cross-contamination risks during food handling.

9. Baron, F., M. Gautier, et al. (1999). "Rapid Growth of *Salmonella* Enteritidis in Egg White Reconstituted from Industrial Egg White Powder."
J Food Prot **62**(6): 585-591.
http://www.ingentaconnect.com/content/iafp/jfp/1999/00000062/00000006/art00004
The aim of this study was to evaluate the consequences of the egg white-drying process on egg white ability to limit *Salmonella* Enteritidis growth in addition to the elucidation of the factors involved. We observed rapid growth of *Salmonella* Enteritidis inoculated in egg white reconstituted from industrial powder in comparison with that observed in liquid egg white collected in the laboratory: *Salmonella* cell counts rose from 10^3 to 10^8 cells/ml of egg white from powder during 24 h incubation at 30°C. This rapid growth was observed in powder from all egg-breaking factories investigated, and it was comparable to that observed in optimum medium (tryptone soy broth). In view of the mechanism of egg white resistance and the major role played by iron availability and by ovotransferrin, we investigated several hypotheses to explain this rapid growth: iron provided during the drying process and/or denaturation of protein (especially ovotransferrin). The rapid growth observed in egg white reconstituted from powder was in relation to egg white protein denaturation and especially ovotransferrin denaturation during powder pasteurization that enhanced the availability of iron necessary for *Salmonella* growth. The major role played by ovotransferrin and iron deficiency on *Salmonella* growth in egg white was illustrated in this study.

10. Bartlett, F. M., J. M. Laird, et al. (1993). "The analysis of egg wash water for the rapid assessment of microbiological quality."
Poult Sci **72**(8): 1584-1591.
http://ps.oxfordjournals.org/content/72/8/1584.long
A total of 101 egg wash water samples from five different egg grading stations in eastern Ontario were analyzed for a variety of physical and chemical variables in an attempt to find a

correlation with total bacterial counts. Temperature, pH, total chlorine, and percentage transmission at 600 nm (%T) were found to be significant variables, and a multiple regression equation was derived that accounted for 65% of the total variation. The equation was used to classify wash water samples as acceptable (< or = 10(5) cfu/mL) or unacceptable and correctly classified 77.2% of the samples. Classification of a second (validation) data set from 58 wash water samples was correctly predicted in 72% of the cases. The predictive value of the equation was especially good for those wash water samples obtained from stations that had used a chlorinated alkaline detergent, 90.4 and 100% for the modeling and validation data, respectively. Maintenance of wash water at recommended levels for temperature and pH (i.e., > or = 40 C and pH > or = 10) was insufficient to ensure bacterial numbers would be < or = 10(5) cfu/mL. Under normal operating conditions a minimum total available chlorine concentration of .45 mg/L should be maintained in wash water to ensure that bacterial numbers are kept at an acceptable level. Monitoring of temperature, pH, total chlorine, and %T will assist in maintaining wash water quality and minimize the number of samples returned to laboratories for microbiological analysis.

11. Bates, C. J. and R. C. Spencer (1995). "Survival of *Salmonella* species in eggs poached using a microwave oven."
J Hosp Infect **29**(2): 121-127.
http://dx.doi.org/10.1016/0195-6701(95)90193-0
The use of microwave ovens is becoming increasingly popular, but there is little data on the bactericidal effect of this mode of cooking. Following a family outbreak of gastroenteritis due to *Salmonella* enteritidis PT4, where eggs poached in a microwave oven were the suspected source, we investigated the survival of *Salmonella* spp. in artifically contaminated eggs cooked in a microwave oven. The survival of six serotypes of *Salmonella* at various inocula were studied, after cooking the eggs using two recognized poaching methods. Salmonellae were readily recovered after cooking if the yolk was still soft, whichever serotype, inoculum or cooking method was used. The survival of organisms was related to the number of organisms present in the raw eggs. The advice regarding eating lightly cooked eggs should be the same whether microwave or more conventional cooking techniques are used.

12. Beatty, M. E., G. Shevick, et al. (2009). "Large *Salmonella* Enteritidis outbreak with prolonged transmission attributed to an infected food handler, Texas, 2002."
Epidemio Infect **137**(Special Issue 03): 417-427.
http://dx.doi.org/10.1017/S0950268808001362
In March 2002, an outbreak of *Salmonella* Enteritidis (SE) infections occurred at a convention centre in Dallas, Texas and continued for 6 weeks. We conducted epidemiological studies, obtained clinical and environmental cultures, and interviewed employees to identify risk factors for infection. From 17 March–25 April 2002, the implicated hotel kitchen catered 41 multi-day conferences attended by 9790 persons. We received 617 illness reports from residents of 46 states. Sauces or items served with sauces were implicated in three cohort studies. SE phage-type 8 was identified as the agent. Eleven food service employees, including one who prepared sauces and salsa, had stool cultures that yielded SE. Although the original source was not determined, prolonged transmission resulted in the largest food handler-associated outbreak reported to date, affecting persons from 46 US states. Transmission ended with implementation of policies to screen food handlers and exclude those whose stool cultures yielded salmonellas.

13. Bell, C. and A. Kyriakides (2002). *Salmonella*: A practical approach to the organism and its control in foods. Oxford, Blackwell Science Ltd.
This book provides an extensive review of this important human pathogen and is not only highly informative but is also an enjoyable read. The authors wisely chose to illustrate the importance of *Salmonella* as a foodborne pathogen and its behaviour not only by reference to published work on scientific studies but also by discussing a range of food poisoning outbreaks and food contamination incidents. The book provides a wealth of information that is of relevance to a range of scientific disciplines and will be particularly interesting to those with an interest in food production and food safety and also in public health microbiology. When one reads this book, it becomes clear that *Salmonella* spp. are really quite remarkable organisms that have the capability of responding to and surviving in a wide variety of different foods and environments. The book also gives a good indication of the ubiquity of this

important human pathogen and also the very wide range of food types that it has contaminated in the past and that will surely become contaminated in the future. The control of salmonellosis requires vigilance by all those involved in the food chain, from farmer to consumer, and this valuable book provides information that will be of help to those involved in the production of safe food and also people intending to enter this field.

14. Berrang, M. E., N. A. Cox, et al. (1991). "Methods for inoculation and recovery of *Salmonella* from chicken eggs."
Poult Sci **70**(11): 2267-2270.
http://www.ncbi.nlm.nih.gov/pubmed/1754543
Various methods of applying inoculum and recovering low numbers of artificially inoculated *Salmonella* Typhimurium on eggs were evaluated. Inoculation methods tested were suspending cells in 1) .85% saline; 2) 1% peptone; 3) sterile chicken fecal paste; or 4) a 1:10 dilution of chicken feces in .85% saline. Sampling methods tested were 1) shell and membrane massage; and 2) mortar and pestle grinding of shells and membranes. The method that yielded the best recovery of low numbers of Salmonella was as follows: 1) apply cell suspension in 1% peptone to egg; 2) sample egg by a hand crush and massage of shell and membranes in 50 mL of buffered peptone; 3) incubate shell and membranes in buffered peptone overnight and then plate onto selective agar. Methods that did not improve sensitivity of recovery included varying the inoculum drying time, addition of FeSO4 or Cleland's reagent to the recovery medium, and varying the temperature of the inoculum to affect penetration.

15. Berrang, M. E., N. A. Cox, et al. (1995). "Efficacy of Ultra Violet Light for Elimination of *Salmonella* on Broiler Hatching Eggs."
J Appl Poult Res **4**(4): 422-429.
http://japr.oxfordjournals.org/content/4/4/422.full.pdf+html
Germicidal UV light at a wavelength of 254 nm was tested as a means to eliminate inoculated *Salmonella* from eggshells. Eggs inoculated with 10^5 cells *Salmonella* were treated with UV light (600 µW/cm2) for 1 to 10 min. When inoculation was made by a drop of suspension on the eggshell surface, UV light treatment significantly lowered the incidence of positive eggs (P≤.05). However, UV light treatment was less effective against *Salmonella* when the inoculum was applied to the egg as a submersion dip. *Salmonella* inoculation placed on the eggshell by a drop of suspension and covered with a fecal smear was not eradicated by the UV light treatment. As few as ten cells of *Salmonella* in feces was sufficient to result in positive eggs following exposure to UV light (600 µW/cm2). Following a fecal smear inoculation with 100 cells, even UV light intensity as high as 1600 µW/cm2 lead to less than 25% reduction in the percentage of eggs positive for *Salmonella*. Continuous exposure of eggs to UV light in the incubator did not affect hatchability.

16. Berrang, M. E., N. A. Cox, et al. (1999). "Bacterial Penetration of the Eggshell and Shell Membranes of the Chicken Hatching Egg: A Review."
J Appl Poult Res **8**(4): 499-504.
http://japr.oxfordjournals.org/content/8/4/499.full.pdf
Bacteria, including human enteropathogens, can penetrate the outer structures of the egg. There are several mechanisms employed by bacteria to gain entry to the egg. The most likely area on the egg to be penetrated is the air cell end, especially when temperature differential and moisture are favorable. The natural defenses that an egg has against such attack are generally not adequate to completely protect the egg from bacteria. The implications and consequences of bacterial penetration of the shell and membranes are serious, including potential dissemination of human pathogens to the hatchery, grow-out flock, and final product. This paper reviews the mechanisms involved in bacterial penetration, methods used to detect penetration, and the stages of modern production which lend themselves to shell penetration and the subsequent potential contamination of many chicks.

17. Blaser, M. J. and L. S. Newman (1982). "A review of human salmonellosis: I. Infective dose."
Rev Infect Dis **4**(6): 1096-1106.
http://www.ncbi.nlm.nih.gov/pubmed/6760337
The notion that large inocula of salmonellae are necessary to induce illness in humans is based on the results of studies involving volunteers. However, investigations of outbreaks of salmonellosis suggest that the infective dose was often low. This incongruity was investigated

by an examination of factors that could affect the infective dose of *Salmonella*, a review of nine studies in which salmonellae were administered to volunteers, and a review of 11 outbreaks of salmonellosis for which the infective doses could be calculated. Determination of the minimal infective doses from studies involving volunteers is limited by the strains used for testing, repeated testing of the same subjects, and the use of too few volunteers at the lower dose levels. In six of the 11 outbreaks, the actual doses ingested were calculated to be less than 10^3 organisms; the outbreaks with higher doses involved very high rates of attack and short periods of incubation. Data presented on median incubation periods during 12 typhoid outbreaks suggest that low doses were involved.

18. Blaser, M. J., E. M. Rafuse, et al. (1981). "An outbreak of salmonellosis involving multiple vehicles."
Am J Epidemiol **114**(5): 663-670.
http://aje.oxfordjournals.org/content/114/5/663.abstract
A biphasic outbreak of salmonellosis affected customers and employees of a restaurant in Maine, in October and November, 1979. *Salmonella* Typhimurium was isolated from 27 customers and nine employees. Ten other individuals were ill but did not have specimens taken for culture, and there were four secondary cases including one affecting a laboratory technician. Fourteen individuals (28%) were hospitalized for up to 45 days, and a total of 416 days of work were lost as a result of the outbreak. Two different food items served at the restaurant at separate times were identified as vehicles. In the first part of the outbreak, 37 persons who consumed either egg-based foods (p = 0.003) or salad (p = 0.006) became ill. In the second phase of the outbreak, in late October and November, nine cases were associated with consumption of inadequately refrigerated salad (p = 0.008), but not with egg-based foods. An infected symptomatic food handler prepared the incriminated salad for some members of this latter group. Use of antacids was also associated with illness (p = 0.03). The occurrence of illness over a seven-week period, the implication of several vehicles, and the demonstration of secondary cases suggest that “sporadic” cases of saimonellosis in the community may be linked.

19. Board, R. G. and N. A. Halls (1973). "The cuticle: A barrier to liquid and particle penetration of the shell of the hen's egg."
Br Poult Sci **14**(1): 69-97.
http://www.tandfonline.com/doi/abs/10.1080/00071667308415999
Synopsis The cuticle of the egg shell prevents water?soluble dyes and carbon black from entering the majority of pores. A small percentage of eggs obtained from a commercial flock had no cuticle and their shells were easily invaded by carbon black. Chemical or physical removal of cuticle resulted in the pores being flooded with water which carried in carbon black. Such eggs did not, however, absorb water at a rate equal to that of eggs from which a piece of shell had been removed. This indicated that the pores without a cap and plug of cuticular material resisted the movement of water. The role of the cuticle and shell in repelling water is discussed and a function akin to that of the plastron of insect eggs proposed.

20. Bolton, D. J., C. J. O'Neill, et al. (2012). "A preliminary study of *Salmonella*, verocytotoxigenic *Escherichia coli*/*Escherichia coli* O157 and *Campylobacter* on four mixed farms."
Zoonoses Public Health **59**(3): 217-228.
http://onlinelibrary.wiley.com/doi/10.1111/j.1863-2378.2011.01438.x/abstract
The aims of this study were to investigate the incidence of *Salmonella*, verocytotoxigenic *Escherichia coli* (VTEC)/*Escherichia coli* O157 and *Campylobacter* on four mixed farms and to characterize the isolates in terms of a range of virulence factors. Eighty-nine composite (five different samples from the same animal species combined) faecal [cattle (24), pigs (14), sheep (4), poultry (4), horses (7), deer (4), dogs (9), rodents (2) and wild birds (20)] samples, 16 composite soil samples plus 35 individual water samples were screened using culture-based, immunomagnetic separation and molecular methods. *Salmonella* was detected in bovine faeces, cattle and poultry house water. *Salmonella* serotypes/phage types included Dublin, Kiel and Typhimurium DT193, and most isolates were spvC, invA and rck positive. The pefA and rck genes were found exclusively in the non-Typhimurium strains, while *Salmonella* Dublin and *Salmonella* Kiel strains carried *Salmonella* genomic island I marker(s). VTEC/*E. coli* O157 were found in deer and dog faeces only. The *E. coli* O157 isolate was an enteroinvasive *E. coli*, while the VTEC isolate was untypable but carried the vt1, eaeA, hlyA,

tir and eptD genes. This article reports the first confirmed carriage of *E. coli* O157 in Irish deer. *Campylobacter* species were not detected over the course of this study. It was concluded that [1] *Salmonella*, VTEC and *Campylobacter* have low (<5%) prevalence or are absent on the farms in this study; [2] water was an important source of bacterial pathogens; [3] both dogs and deer may act as a source of pathogenic *E. coli* and [4] key virulence and resistance determinants are widespread in farm *Salmonella* strains. This study highlights the need to control water as a source of pathogens and suggests that the domestic pets and deer should be considered in any farm risk assessment.

21. Boqvist, S., I. Hansson, et al. (2003). "*Salmonella* isolated from animals and feed production in Sweden between 1993 and 1997."
Acta Vet Scand **44**(4): 181-197.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1831546/
This paper presents *Salmonella* data from animals, feedstuffs and feed mills in Sweden between 1993 and 1997. During that period, 555 isolates were recorded from animals, representing 87 serotypes. Of those, 30 serotypes were found in animals in Sweden for the first time. The majority of all isolates from animals were *S*. Typhimurium (n = 91), followed by *S*. Dublin (n = 82). There were 115 isolates from cattle, 21 from broilers, 56 from layers and 18 from swine. The majority of these isolates were from outbreaks, although some were isolated at the surveillance at slaughterhouses. The number of isolates from the feed industry was similar to that of the previous 5-year period. Most of those findings were from dust and scrapings from feed mills, in accordance with the HACCP programme in the feed control programme. It can be concluded that the occurrence of *Salmonella* in animals and in the feed production in Sweden remained favourable during 1993–97.

22. Borda, D., M. R. Thomas, et al. (2014). "Food safety practices in European TV cooking shows."
Br Food J **116**(10): 1652-1666.
http://www.emeraldinsight.com/doi/abs/10.1108/BFJ-12-2013-0367
The purpose of this paper is to determine how well cooking shows promote safe food handling via TV and to suggest their use for providing good hygiene and good cooking practices examples for consumers. Design/methodology/approach – Principal component analysis was applied for the multivariate statistical analysis of the cooking shows, the components being: personal hygiene, cross-contamination, cooking and storing practices and risk communication. Data were collected via a questionnaire special designed for the purpose of the study. The positive attributes were converted into numbers using a nine-point Likert scale. This conversion enabled ranking of the cooking shows as a function of the total results obtained and considering the best show as the one with the maximum score attained. Findings – Evaluation of cooking practices by food safety professionals highlighted the most frequent safety errors and poor practices that are disseminated by the TV shows. Practical implications – While the repetition of good food handling and cooking practices risks antagonizing viewers, an increase in occasional emphasis of good hygiene would be of benefit to domestic viewers and potentially improve food safety practices among the public. Originality/value – This is the first study that gives an European perspective on presentation of safety practices during food handling and preparation in a range of TV cooking shows as it examines 19 such shows broadcasted in six European countries over three months. Adherence to food safety standards and introduction of a star rating system for safety practices in TV cooking shows is proposed.

23. Borland, E. D. (1975). "*Salmonella* infection in poultry."
Vet Rec **97**(21): 406-408.
http://www.ncbi.nlm.nih.gov/pubmed/766359
Poutry may acquire a wide range of *Salmonella* serotypes from various sources including feedingstuffs, breeding flocks, rodents, wild birds and other vectors. Clinical disease is uncommon, but all infections are of importance as potential sources of food poisoning in man.

24. Bradford, M. A., T. J. Humphrey, et al. (1997). "The cross-contamination and survival of *Salmonella* Enteritidis PT4 on sterile and non-sterile foodstuffs."
Lett Appl Microbiol **24**(4): 261-264.
http://onlinelibrary.wiley.com/doi/10.1046/j.1472-765X.1997.00127.x/pdf

The ability of two strains of *Salmonella* Enteritidis PT4 to cross-contaminate from inoculated egg droplets on surfaces onto melon or beef (sterile or non-sterile) was investigated. When the foods were placed on these surfaces where egg droplets were still wet, cross-contamination occurred within 1 s onto every piece of food. It took at least 1 min for all the food pieces to be contaminated when egg droplets had been allowed to dry. Both strains were capable of rapid growth on melon and beef (sterile or non-sterile) at 20 degrees C, but growth rates on beef appeared to be slowed by pre-exposure to either 4 or -18 degrees C.

25. Brake, J. and B. W. Sheldon (1990). "Effect of a quaternary ammonium sanitizer for hatching eggs on their contamination, permeability, water loss, and hatchability."
Poult Sci **69**(4): 517-525.
http://www.ncbi.nlm.nih.gov/pubmed/2356172
The present study tested the biocidal effectiveness of the quaternary ammonium Hatching Egg Sanitizer Spray (HES Spray) in disinfecting broiler hatching eggs and in terms of its effects on eggshell permeability, water loss, and hatchability. The application of HES at a 1.5% or a 3.0% concentration resulted in significant reductions in the total aerobic counts on the egg surface of 98.1% and 99.9%, respectively, within 30 min of application. Molds and yeasts were significantly reduced by 3.0% HES at 14 days of incubation. Significant reductions in total aerobic counts on the egg surface due to 1.5% and 3.0% HES were also observed on eggs that were allowed to "sweat." The 3.0% HES concentration also reduced coliforms on egg surfaces. Hatchability of fertile eggs from a 32-wk-old flock was significantly increased, over 6.0%, by spraying 1.5% or 3.0% HES in comparison to controls that were not sprayed, with no significant difference in hatchability due to treatment observed in eggs from flocks that were 36, 42, 46, or 62 wk of age. This change in hatchability associated with spraying HES may be due to a change in eggshell permeability (respiration) caused by an interaction of HES with the eggshell cuticle.

26. Broennum Pedersen, T., J. Elmerdahl Olsen, et al. (2008). "Persistence of *Salmonella* Senftenberg in poultry production environments and investigation of its resistance to desiccation."
Avian Pathol **37**(4): 421-427.
http://www.tandfonline.com/doi/pdf/10.1080/03079450802216561
Most *Salmonella* serovars, including *Salmonella* enterica subsp. enterica serovar *Senftenberg* (*S.* Senftenberg), are tolerant to desiccation and able to colonize and persist in feed mills. In addition, they may survive cleaning and disinfection procedures used on poultry farms. The present study was conducted to investigate the survival of *S.* Senftenberg in broiler parent stock farms and broiler farms. The isolates from one of the parent stock farms investigated only differed by a single band in fluorescent amplified fragment-length polymorphism analysis and had identical pulsed-field gel electrophoresis profiles, indicating that a *S.* Senftenberg clone had persisted for more than 2 years, despite cleaning, disinfection, desiccation and depopulation, and was subsequently able to infect *Salmonella*-free layers. Isolates from the same house on a different broiler parent stock farm were found to be identical by amplified fragment-length polymorphism analysis and pulsed-field gel electrophoresis although the farm tested negative for *Salmonella* 55 times over a period of 18 months between the two positive samplings. An assay was developed to investigate the survival of 34 *S.* Senftenberg isolates during desiccation at approximately 38% relative humidity. On average, the viability of *S.* Senftenberg isolates decreased by 1000-fold over 35 days. The persistent clones were no more resistant to desiccation than the other isolates investigated. However, *S.* Senftenberg was more resistant to desiccation than an isolate of Pantoea agglomerans commonly found on poultry feed-processing lines. This study demonstrates the risk of persistence of feed-associated serovars such as *S.* Senftenberg.

27. Brown, W. E., R. C. Baker, et al. (1966). "The microbiology of cracked eggs."
Poult Sci **45**(2): 284-287.
http://ps.oxfordjournals.org/content/45/2/284.full.pdf+html
Although a great deal of research has been published dealing with various aspects of egg spoilage by bacteria, a very limited portion of this has been devoted to cracked eggs. McNally (1953) found the rate of infection of cracked eggs was more affected by temperature and humidity than that of normal eggs. Miller and Crawford (1953) reported that 7 out of 50 commercial checks, selected to exclude thin shells and leakers, contained an average of 7 ×

10^6 bacteria per ml. The present investigation was initiated to determine the relative susceptibility of cracked and normal eggs to bacterial invasion and to determine some of the factors which might predispose cracked eggs to rapid spoilage. All eggs used in this study were from a single strain of Single Comb White Leghorns. Unless stated to the contrary, eggs were received from the farm one day post lay.

28. Callaway, T. R., T. S. Edrington, et al. (2014). "Isolation of *Escherichia coli* O157:H7 and *Salmonella* from migratory brown-headed cowbirds (*Molothrus ater*), common Grackles (*Quiscalus quiscula*), and cattle egrets (*Bubulcus ibis*)."
Foodborne Pathog Dis **11**(10): 791-794.
http://online.liebertpub.com/doi/abs/10.1089/fpd.2014.1800?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub%3Dpubmed
Zoonotic enteric pathogenic bacteria can live in the intestinal tract of birds and can be transmitted to food animals or humans via fecal contact. In the present study, cecal samples were collected from 376 migratory birds from species often associated with cattle during the fall migration in the Central Flyway of the United States. Brown-headed cowbirds (n=309, *Molothrus ater*), common grackles (n=51, *Quiscalus quiscula*), and cattle egrets (n=12, Bubulcus ibis) contained foodborne pathogenic bacteria in their ceca. *Salmonella* enterica was isolated from 14.9% of all samples, and *Escherichia coli* O157:H7 from 3.7%. *Salmonella* serotypes isolated included the following: Muenster, Montevideo, and Typhimurium. Our data suggest that migratory birds associated with cattle could be a vector for zoonotic enteric pathogenic bacteria to be disseminated across long distances.

29. Caudill, A. B., P. A. Curtis, et al. (2010). "The effects of commercial cool water washing of shell eggs on Haugh unit, vitelline membrane strength, aerobic microorganisms, and fungi."
Poult Sci **89**(1): 160-168.
http://ps.oxfordjournals.org/content/89/1/160.long
Current egg washing practices use wash water temperatures averaging 49 degrees C and have been found to increase internal egg temperature by 6.7 to 7.8 degrees C. These high temperatures create a more optimal environment for bacterial growth, including *Salmonella* Enteritidis if it is present. *Salmonella* Enteritidis is the most common human pathogen associated with shell eggs and egg products. Its growth is inhibited at temperatures of 7.2 degrees C and below. The objective of this study was to determine if commercially washing eggs in cool water would aid in quickly reducing internal egg temperature, preserving interior egg quality, and slowing microbial growth. During 3 consecutive days, eggs were washed using 4 dual-tank wash water temperature schemes (HH = 49 degrees C, 49 degrees C; HC = 49 degrees C, 24 degrees C; CC = 24 degrees C, 24 degrees C; CH = 24 degrees C, 49 degrees C) at 2 commercial processing facilities. A 10-wk storage study followed, in which vitelline membrane strength, Haugh unit, and aerobic microorganisms and fungi (yeasts and molds) were monitored weekly. As storage time progressed, average Haugh unit values declined 14.8%, the average force required to rupture the vitelline membrane decreased 20.6%, average numbers of bacteria present on shell surfaces decreased 11.3%, and bacteria present in egg contents increased 39.5% during storage. Wash water temperature did not significantly affect Haugh unit values, vitelline membrane strength, or the numbers of aerobic microorganisms and fungi within the shell matrices of processed eggs. Results of this study indicate that incorporating cool water into commercial shell egg processing, while maintaining a pH of 10 to 12, lowers postprocessing egg temperatures and allows for more rapid cooling, without causing a decline in egg quality or increasing the presence of aerobic microorganisms and fungi for approximately 5 wk postprocessing.

30. Chaidez, C., M. Soto-Beltran, et al. (2014). "Reduction of risk of *Salmonella* infection from kitchen cleaning clothes by use of sodium hypochlorite disinfectant cleaner."
Lett Appl Microbiol **59**(5): 487-492.
http://dx.doi.org/10.1111/lam.12321
The objective of this study was to evaluate the reduction of infection risk due to exposure to *Salmonella* sp. in kitchen cleaning clothes by the use of a bleach (sodium hypochlorite) cleaner utilizing a continuous-time dynamic exposure model. The only route of exposure considered was hand contamination during cloth use. The occurrence and numbers of *Salmonella* was studied in 60 homes over a 6-week period in which half disinfected kitchen cleaning clothes with a sodium hypochlorite based disinfectant cleaner. This study

demonstrated that a significant risk exists for *Salmonella* infection from kitchen cleaning clothes in Mexican homes and that this risk can be reduced by almost 100-fold by soaking cleaning clothes in a bleach product. The risks of infection and illness could likely be further reduced by developing a more effective procedure for reducing *Salmonella* in cleaning clothes treated twice a day with a sodium hypochlorite disinfectant (i.e. longer soaking time) or using a greater concentration of the disinfectant.
Significance and Impact of the Study Hygiene intervention is a key strategy to reduce the potential risk of disease-causing micro-organisms in households. There is a lack of understanding of the human health risk associated with the use of contaminated kitchen cleaning cloths. The study used a quantitative microbial risk assessment to estimate the risk associated with the use of kitchen cleaning clothes by using disinfectant products. The results showed that the use of prescribe protocols can reduce the risk of *Salmonella* infections in household kitchens.

31. Chalghoumi, R., A. Thewis, et al. (2009). "Adhesion and growth inhibitory effect of chicken egg yolk antibody (IgY) on *Salmonella* enterica serovars Enteritidis and Typhimurium in vitro." Foodborne Pathog Dis **6**(5): 593-604.
http://www.ncbi.nlm.nih.gov/pubmed/19388827
The protective effects of powder preparation of egg yolk immunoglobulin Y (IgY), specific to *Salmonella* Enteritidis and *Salmonella* Typhimurium outer membrane proteins (OMP), against these two *Salmonella* sp. serovars were investigated in vitro in two different assays: adhesion-prevention and growth-inhibition. The adhesion-prevention assay was conducted using polarized monolayers of the human intestinal epithelial Caco-2 cell line. First, the conditions of *Salmonella* adherence to Caco-2 cells were optimized, and interferences of bacteria with the transepithelial electrical resistance (TER) of fully differentiated Caco-2 cell monolayers and the lactate dehydrogenase release upon exposure of the cells to *Salmonella* were evaluated. Both *Salmonella* sp. serovars were able to adhere to Caco-2 cells and decreased TER. Results from the adhesion-prevention assay demonstrated that specific IgY reduced the decrease in TER of the infected Caco-2 cell monolayers and blocked the *Salmonella* sp. adhesion in a concentration-dependent manner ($p < 0.05$). Nonspecific IgY also exhibited an inhibitory effect on these two parameters, but to a lesser extent than that of the specific IgY ($p < 0.05$). The protective effect of nonspecific IgY could be attributed to the low-density lipoprotein component of the water-soluble fraction of egg yolks that may not have been eliminated during ultrafiltration. The growth-inhibition assay revealed that specific IgY had an inhibitory effect on the bacterial growth, markedly during the late exponential phase, whereas nonspecific IgY failed to do so. Taken together, these results suggest that the in vitro growth inhibitory effect of specific IgY on *Salmonella* spp. resulted from the specific binding activity of these IgY to *Salmonella* sp. OMP. Passive immunization with *Salmonella* sp. OMP-specific IgY could thus be useful to prevent *Salmonella* colonization in broiler chickens and the subsequent carcass contamination during processing.

32. Chemaly, M., A. Huneau-Salaun, et al. (2009). "Isolation of *Salmonella enterica* in laying-hen flocks and assessment of eggshell contamination in France." J Food Prot **72**(10): 2071-2077.
http://www.ncbi.nlm.nih.gov/pubmed/19833029
The present investigation was conducted in conjunction with the European Union baseline study for the estimation of *Salmonella* prevalence in laying-hen flocks. It aimed at evaluating eggshell contamination in farms positive for *Salmonella*, characterizing the genetic patterns of *Salmonella* strains and identifying the factors associated with *Salmonella* contamination of eggshells. For this purpose, a total of 4,200 eggs were collected from 28 positive flocks and analyzed according to draft Annex D of International Organization for Standardization Method 6579. Molecular characterization of the *Salmonella* strains was obtained by the pulsed-field gel electrophoresis method with two restriction enzymes, XbaI and BlnI. The relationship between the presence of *Salmonella* on eggshells and rearing practices was studied by using multiple correspondence analysis. Results showed that 39.3% of the positive flocks had at least one positive eggshell, with a total of 1.05% of eggshells testing positive for *Salmonella*. We detected the same serovars on samples taken from the farm and from eggshells within a given flock, with isolates sharing the same genetic pattern in 7 of 11 flocks. Eggshells tested positive for *Salmonella* in flocks (i) located where delivery trucks pass near air entrances of the poultry house, (ii) with high holding capacity (>30,000 laying hens), and (iii) with more

than five positive samples coming from the farm environment, as well as in cases of flocks with a maximum egg-laying rate of >96% and in cases where farmers worked in other animal production. This study provided valuable information that could be used for risk management and risk assessment studies.

33. Chen, H., R. C. Anantheswaran, et al. (2002). "Effect of rapid cooling of shell eggs on microcrack development, penetration of *Salmonella* Enteritidis, and eggshell strength." J Food Process Preserv **26**(1): 57-73. http://dx.doi.org/10.1111/j.1745-4549.2002.tb00477.x
Eggs were subjected to cryogenic cooling treatments using liquid CO_2 or liquid N_2. In order to minimize the thermal stress in eggshells due to rapid cooling, a two-stage air-cooling method was also evaluated in this study. Eggs were cooled from an initial temperature of 25C to approximately 7C. It was found that cooling produced microcracks on eggshells. However, rapid cooling did not increase the penetration of *Salmonella enterica* serovar Enteritidis (*Salmonella* Enteritidis) into egg contents. When egg contents alone were sampled for *Salmonella* Enteritidis, extending the immersion time from 24 to 48 h significantly ($P < 0.01$) increased the penetration of *Salmonella* Enteritidis from 5.0 to 25.0%. When egg contents together with eggshells were sampled, *Salmonella* Enteritidis was detected in 100% of the egg samples at the above two time intervals. There were no significant differences ($P > 0.05$) in the eggshell strength between control (no cooling) and cooling treatments, indicating that cooling did not weaken eggshell strength.

34. Chia, T. W., R. M. Goulter, et al. (2009). "Attachment of different *Salmonella* serovars to materials commonly used in a poultry processing plant." Food Microbiol **26**(8): 853-859. http://www.ncbi.nlm.nih.gov/pubmed/19835771
Salmonella can adhere to poultry and food contact surfaces and persist to cause diseases. Adhesion of *Salmonella* Sofia (n = 14), *S.* Typhimurium (n = 6), *S.* Infantis (n = 3) and *S.* Virchow (n = 2) to Teflon, stainless steel, glass, rubber and polyurethane were assayed using epifluorescence microscopy. Surface free energies of bacteria and materials were calculated using contact angle values and interfacial free energy between isolates and materials determined. Surface roughness of the materials was analysed using atomic force microscopy. *S.* Sofia isolates adhered in higher numbers ($P < 0.05$) to all materials compared to other serovars. The mean number of cells of *S.* Sofia isolates attaching to Teflon were significantly higher ($P < 0.05$) compared to all materials except stainless steel ($P > 0.05$). Mean roughness values ranged from 82.26 nm (Teflon) to 1.34 nm (glass). Correlations between the apolar component of the surface free energy of materials (gamma(S)(LW)) and bacterial adhesion ($R(2) = 0.80$), and between gamma(S)(LW) and the surface roughness of the materials ($R(2) = 0.71$) were found. Materials more positive in interfacial free energies had the highest number of adhering bacteria. Generalised surface property measurements were found to be useful in characterising Salmonella attachment but the degree of variability in results suggests that other factors, such as flagella or membrane proteins, could also contribute.

35. Chinivasagam, H. N., M. Redding, et al. (2010). "Presence and incidence of food-borne pathogens in Australian chicken litter." Br Poult Sci **51**(3): 311-318. http://www.ncbi.nlm.nih.gov/pubmed/20680865
1. Litter samples were collected at the end of the production cycle from spread litter in a single shed from each of 28 farms distributed across the three Eastern seaboard States of Australia.
2. The geometric mean for *Salmonella* was 44 Most Probable Number (MPN)/g for the 20 positive samples. Five samples were between 100 and 1000 MPN/g and one at 10(5) MPN/g, indicating a range of factors are contributing to these varying loads of this organism in litter.
3. The geometric mean for *Campylobacter* was 30 MPN/g for the 10 positive samples, with 7 of these samples being <100 MPN/g. The low prevalence and incidence of *Campylobacter* were possibly due to the rapid die-off of this organism.
4. *E. coli* values were markedly higher than the two key pathogens (geometric mean 20 x 10(5) colony forming units (cfu)/g) with overall values being more or less within the same range across all samples in the trial, suggesting a uniform contribution pattern of these organisms in litter.

5. *Listeria monocytogenes* was absent in all samples and this organism appears not to be an issue in litter.
6. The dominant (70% of the isolates) *Salmonella* serovar was *S.* Sofia (a common serovar isolated from chickens in Australia) and was isolated across all regions. Other major serovars were *S.* Virchow and *S.* Chester (at 10%) and *S.* Bovismorbificans and *S.* Infantis (at 8%) with these serovars demonstrating a spatial distribution across the major regions tested.
7. There is potential to re-use litter in the environment depending on end use and the support of relevant application practices and guidelines.

36. Chousalkar, K. K., P. Flynn, et al. (2010). "Recovery of *Salmonella* and *Escherichia coli* from commercial egg shells and effect of translucency on bacterial penetration in eggs." Int J Food Microbiol **142**(1-2): 207-213. http://www.ncbi.nlm.nih.gov/pubmed/20663580
This experiment was conducted to study the prevalence of *Salmonella* and *Escherichia coli* (*E. coli)*. from the surface of egg shells, egg shell membranes or pores, and internal contents from unwashed eggs collected from commercial caged layer farms in Australia. Egg shell swabs, shell crush and egg internal contents (yolk and albumen) of an individual egg were processed for bacteriological examination. *Salmonella* spp. were not detected from any of the egg shell surfaces, egg shell crush or egg internal contents. Thirty five *E. coli* isolates were isolated from the egg shell surface. Ten *E. coli* strains were also isolated from shell crush. However, the internal contents of eggs appeared to be sterile. Polymerase chain reaction was performed on forty-five *E. coli* isolates using primers for heat stable enterotoxin genes A and B (STa and STb) and also for colicin V gene (cvaC). STa gene was detected in four *E. coli* isolates isolated from egg shell surfaces. All the *E. coli* isolates were negative for STb and cvaC genes. These data provide useful information regarding the prevalence of virulent *E. coli* and *Salmonella* spp. on and in unwashed eggs collected from layer farms. These data also suggest that unwashed eggs collected from caged layer farms are unlikely to be sources of *Salmonella* outbreaks. Egg shell translucency could be due to changes in the mammillary layer and mamillary cores during the early phases of egg shell formation and has the potential to increase the incidence of microcracks in egg shells, and hence, may be responsible for bacterial penetration. There was a significant correlation between egg shell translucency and egg shell penetration by *Salmonella* Infantis and *E. coli*. Both strains of bacteria were able to penetrate the translucent egg shells even at very low doses. The penetration, however, was hindered in both translucent and non translucent eggs at 4 degrees C, as compared with room temperature which highlights the importance of storage of eggs at refrigerated temperatures.

37. Chousalkar, K. K. and J. R. Roberts (2012). "Recovery of *Salmonella* from eggshell wash, eggshell crush, and egg internal contents of unwashed commercial shell eggs in Australia." Poult Sci **91**(7): 1739-1741. http://ps.oxfordjournals.org/content/91/7/1739.full.pdf
The experiment was conducted to study the prevalence of *Salmonella* spp. on the eggshell surface, eggshell membranes or pores, and in egg internal contents from unwashed eggs collected from commercial caged layer farms in Australia. Eggshell rinsate, shell crush, and egg internal contents (yolk and albumen) of eggs were processed for *Salmonella* spp. *Salmonella* Infantis and *Salmonella* subspecies 1, serotype 4,12:d were isolated from the eggshell surface. *Salmonella* spp. were not isolated from any eggshell crush or egg internal contents. It would appear that the occurrence of *Salmonella* in the Australian egg industry is low.

38. Clay, C. E. and R. G. Board (1991). "Growth of *Salmonella* Enteritidis in artificially contaminated hens' shell eggs." Epidemiol Infect **106**(2): 271-281. http://www.ncbi.nlm.nih.gov/pubmed/2019298
The effect of some factors on the growth of *Salmonella* Enteritidis phage type 4 in artificially contaminated shell eggs was investigated. *Salmonella* Enteritidis was found to be resistant to the antimicrobial properties of the albumen. Growth occurred on storage at 25 degrees C but not at 4 or 10 degrees C. The rate and extent of infection was influenced by the size of inoculum, the site of contamination relative to yolk movement, and the presence of iron in the inoculum.

39. Coloe, P. J., M. R. Alderton, et al. (1995). "Aromatic vitamin-dependent Salmonellae as vaccines in food animals: efficacy and persistence."
Dev Biol Stand **84**: 263-267.
http://www.ncbi.nlm.nih.gov/pubmed/7796963

40. Coloe, P. J., J. Taplin, et al. (1984). "The distribution of specific phage types of *Salmonella* Typhimurium in chickens in Australia."
J Hyg (Lond) **92**(2): 177-182.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2129249/pdf/jhyg00017-0050.pdf
The distribution of specific phage types of *Salmonella* Typhimurium within the Australian chicken industry has been studied and documented on an Australia-wide and state-by-state basis. A total of 1799 strains of *S*. Typhimurium were obtained from Australia-wide sources and phage typing categorized 1498 of these isolates into 30 distinct phage types, with the remaining 301 strains untypable. Five phage types, 6, 26, 31, 135 and 179, accounted for 76% of the total strains typed, with the remaining 24% of strains being distributed among 25 phage types. Of the major phage types, type 31 was restricted to Victoria and Western Australia, but the other types were distributed throughout Australia. In addition, the antibiotic resistance pattern of the various phage types was determined and only five of the 30 phage types showed appreciable levels of resistance.

41. Corcoran, M., D. Morris, et al. (2014). "Commonly used disinfectants fail to eradicate *Salmonella enterica* biofilms from food contact surface materials."
Appl Environ Microbiol **80**(4): 1507-1514.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3911063/
Salmonellosis is the second most common cause of food-borne illness worldwide. Contamination of surfaces in food processing environments may result in biofilm formation with a risk of food contamination. Effective decontamination of biofilm-contaminated surfaces is challenging. Using the CDC biofilm reactor, the activities of sodium hypochlorite, sodium hydroxide, and benzalkonium chloride were examined against an early (48-h) and relatively mature (168-h) *Salmonella* biofilm. All 3 agents result in reduction in viable counts of *Salmonella*; however, only sodium hydroxide resulted in eradication of the early biofilm. None of the agents achieved eradication of mature biofilm, even at the 90-min contact time. Studies of activity of chemical disinfection against biofilm should include assessment of activity against mature biofilm. The difficulty of eradication of established *Salmonella* biofilm serves to emphasize the priority of preventing access of *Salmonella* to postcook areas of food production facilities.

42. Corry, J. E., V. M. Allen, et al. (2002). "Sources of *Salmonella* on broiler carcasses during transportation and processing: modes of contamination and methods of control."
J Appl Microbiol **92**(3): 424-432.
http://www.ncbi.nlm.nih.gov/pubmed/11872117
AIMS: The prevalence and types of *Salmonella* in broiler chickens during transportation and during slaughter and dressing were studied. This was part of a comprehensive investigation of salmonellas in two UK poultry companies, which aimed to find the origins and mechanisms of salmonella contamination.
METHODS AND RESULTS: Salmonellas were isolated using cultural methods. Serovars of Salmonella detected during rearing were usually also found in a small proportion of birds on the day of slaughter and on the carcasses at various points during processing. There was little evidence of salmonellas spreading to large numbers of carcasses during processing. Many serovars found in the feedmills or hatcheries were also detected in the birds during rearing and/or slaughter. Transport crates were contaminated with salmonellas after washing and disinfection.
CONCLUSIONS: Prevalence of salmonellas fell in the two companies during this survey. A small number of serovars predominated in the processing plants of each company. These serovars originated from the feed mills. Reasons for transport crate contamination were: (1) inadequate cleaning, resulting in residual faecal soiling; (2) disinfectant concentration and temperature of disinfectant too low; (3) contaminated recycled flume water used to soak the crates.

SIGNIFICANCE AND IMPACT OF THE STUDY: Efforts to control salmonella infection in broilers need to concentrate on crate cleaning and disinfection and hygiene in the feed mills.

43. Cox, J. M. and A. Pavic (2010). "Advances in enteropathogen control in poultry production." J Appl Microbiol **108**(3): 745-755.
http://www.ncbi.nlm.nih.gov/pubmed/19702864
Poultry meat has been associated frequently and consistently with the transmission of enteric pathogens, including *Salmonella* and *Campylobacter*. This association has resulted in the development of HACCP-based intervention strategies. These strategies (hurdles) begin with elite breeder flocks and filter down the production pyramid. These hurdles include those already established, such as biosecurity, vaccination, competitive exclusion, pre- and probiotics, feed and water control, and those more experimental, such as bacteriophage or immunoglobulin therapy. The reduction in enteropathogens entering the processing plant, which employs critical control points, further reduce the exposure of consumers to these organisms. The synergistic application of hurdles will result in an environment that is restrictive and detrimental to enteropathogen colonization and contamination.

44. Cox, N. A., M. E. Berrang, et al. (2000). "*Salmonella* penetration of egg shells and proliferation in broiler hatching eggs--a review." Poult Sci **79**(11): 1571-1574.
http://ps.oxfordjournals.org/content/79/11/1571.long
The presence of salmonellae in fertile broiler hatching eggs has been clearly identified as a critical control point in the salmonellae contamination of broiler chickens. This paper reviews the published research studies on a) the penetration and proliferation of salmonellae in hatching eggs, b) the consequences of this contamination on the contamination of the final product, and c) the egg's defenses against invading salmonellae. A better understanding of the material in this review paper will assist poultry researchers and the poultry industry in continuing to make progress in reducing and eliminating salmonellae from fertile hatching eggs, hatcheries, and breeder flocks.

45. Crump, J. A., P. M. Griffin, et al. (2002). "Bacterial contamination of animal feed and its relationship to human foodborne illness." Clin Infect Dis **35**(7): 859-865.
http://cid.oxfordjournals.org/content/35/7/859.long
Animal feed is at the beginning of the food safety chain in the "farm-to-fork" model. The emergence of variant Creutzfeldt-Jakob disease has raised awareness of the importance of contaminated animal feed, but less attention has been paid to the role of bacterial contamination of animal feed in human foodborne illness. In the United States, animal feed is frequently contaminated with non-Typhi serotypes of *Salmonella* enterica and may lead to infection or colonization of food animals. These bacteria can contaminate animal carcasses at slaughter or cross-contaminate other food items, leading to human illness. Although tracing contamination to its ultimate source is difficult, several large outbreaks have been traced back to contaminated animal feed. Improvements in the safety of animal feed should include strengthening the surveillance of animal feed for bacterial contamination and integration of such surveillance with human foodborne disease surveillance systems. A Hazard Analysis and Critical Control Point program should be instituted for the animal feed industry, and a *Salmonella*-negative policy for feed should be enforced.

46. Daughtry, B., J. Sumner, et al. (2005). National food safety risk profile of eggs and egg products.
https://www.aecl.org/assets/www.aecl.org/outputs/SAR-47A-Final-Report.pdf
This project aimed to conduct a through chain food safety risk profile for the Australian egg industry. Risk profiling is now recognised as an important first step that is essential for effective food safety risk management. It has recently been defined as ‘a description of a food safety problem and its context developed for the purpose of identifying those elements of a hazard or risk that are relevant to risk management decisions’ (Codex Alimentarius Commission). Risk profiling involves the systematic collection of information needed to make a decision on what will be done next and where resources should be allocated to more detailed scientific assessment. The risk profiling process typically provides information on: the hazard, exposure to the hazard, adverse health effects, public health surveillance information,

appropriate options for control, and other information relevant to risk management decision-making. The provision of a comprehensive description of the food safety problem associated with the pathogen(s):commodity combination(s) from farm to fork is recommended. This process is increasingly being adopted across Australia by jurisdictions responsible for protecting public health. This report identifies: • hazards that entered any point of the food chain for eggs and egg products produced in Australia and ranks them in terms of health risk to the consumer • hazards of potentially high risk where too little information exists for a confident ranking of risk and "what if" scenarios raised by risk managers during the expert consultations • potential management strategies for the identified high risk hazards • product/pathogen combinations in which further risk analysis might be required by risk managers. An extensive process of expert consultations between risk managers and assessors was used to guide management of the project. This was to ensure the outputs adequately address the major food safety concerns and were provided in a timely manner to support current and pending risk management processes.

47. Davies, R. and M. Breslin (2004). "Observations on *Salmonella* contamination of eggs from infected commercial laying flocks where vaccination for *Salmonella enterica* serovar Enteritidis had been used."
Avian Pathol **33**(2): 133-144.
http://www.ncbi.nlm.nih.gov/pubmed/15276979
Eggs were collected monthly from 12 cage-layer flocks on four farms where *Salmonella* Enteritidis was present in vaccinated flocks despite vaccination with an *S.* Enteritidis bacterin. Where possible, hens were also taken for culture at the end of the laying period, and faecal and environmental samples were taken from the laying houses before and after cleaning and disinfection. Twenty-four batches of six egg shells from the 13 652 tested (0.18% [0.11 to 0.26 CI(95)] single egg equivalent) were positive for *S.* Enteritidis and 54 (0.40% [0.30 to 0.52 CI(95)] single egg equivalent) for other serovars. Six batches of 13 640 (0.04% [0.02 to 0.10 CI95] single egg equivalent) egg contents, bulked in six egg pools, contained *S.* Enteritidis and three batches contained other serovars. In addition three further batches contained *S.* Enteritidis in both contents and shells, and two other batches contained other serovars in both. The total level of contamination by *S.* Enteritidis of both contents and shells found in vaccinated flocks was therefore 33 batches/13 682 eggs(0.24% [0.17 to 0.34 CI(95)] single egg equivalent). The total of contamination for any *Salmonella* serovar was 92 batches/13 682 eggs (0.68% [0.55 to 0.84 CI(95)] single egg equivalent). These results contrast with the findings of testing of eggs from three unvaccinated flocks prior to this study where 21 batches of egg shells from a total of 2101 eggs (1.0% [0.63 to 1.56 CI(95)] single egg equivalent) and six batches of contents from 2051 eggs (0.29% [0.11 to 0.64 CI95] single egg equivalent) were contaminated with *S.* Enteritidis. *S.* Enteritidis was found in 67/699 (9.6%) of vaccinated spent hens and 64/562 (11.4%) of bulked fresh faecal samples taken from laying houses. Failure to adequately clean and disinfect laying houses and to control mice appeared to be a common feature on the farms.

48. Davies, R. H. and M. Breslin (2003). "Investigation of *Salmonella* contamination and disinfection in farm egg-packing plants."
J Appl Microbiol **94**(2): 191-196.
http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2672.2003.01817.x/full
AIMS: As part of a field-based study of the distribution and persistence of *Salmonella* infection on commercial egg-laying farms, sampling was carried out on one or more occasions in egg-packing areas of 12 farms infected with *Salm*. Enteritidis.
METHODS AND RESULTS: Salmonellas were isolated by cultural methods. Contamination was common, with *Salmonella* being found in 23.1% of floor swab samples, 30.8% of grading tables, 23.1% of conveyor belts or rollers and 23.8% of candlers. Four farms were sampled after cleaning and disinfection of packing plants had been carried out on the previous day, and residual contamination was found on 6.9% of samples from grading tables, 16.0% holding/sorting tables, 12.6% of conveyors or rollers, 16.7% of vacuum egg lifters, 21.4% of floor surfaces and 5.0% of egg store floor surfaces. Sterilized eggs passed through five farm packing plants showed a contamination rate of at least 16/5,948 (0.3%) egg passages. CONCLUSIONS: It is apparent that contamination in egg-packing plants may be a significant contributory factor to external contamination of shell eggs, and improved methods of cleaning and disinfecting egg-handling equipment are required.

SIGNIFICANCE AND IMPACT OF THE STUDY: The presence of *Salmonella* contamination in egg-packing plants presents a contamination hazard for eggs from *Salmonella*-free flocks. Samples from equipment in the packing plant could also be used for screening for detection of *Salmonella* in the throughout of the plant.

49. Davies, R. H. and C. Wray (1996). "Persistence of *Salmonella* Enteritidis in poultry units and poultry food."
Br Poult Sci **37**(3): 589-596.
http://www.ncbi.nlm.nih.gov/pubmed/8842466
1. Studies on the survival of *Salmonella* Enteritidis in poultry units and food were carried out over a two-year period.
2. The organism persisted for at least one year in an empty trial house at the laboratory in which naturally-infected broiler breeder birds had previously been housed. A similar survival period was seen in a building which had housed an infected layer breeder flock, although infection was not detected in a subsequent pullet flock.
3. *Salmonella* Enteritidis was also frequently found surviving outside poultry houses in small pockets of litter and fan dust which had been left after cleansing and disinfection of the site. On some poultry units *S*. Enteritidis was also found in wild bird droppings.
4. *Salmonella* contamination appeared to persist preferentially in association with dust particles swept from the floor and in food troughs and *S.* Enteritidis survived at least 26 months in artificially contaminated poultry food.

50. Davies, R. H. and C. Wray (1996). "Studies of contamination of three broiler breeder houses with *Salmonella* Enteritidis before and after cleansing and disinfection."
Avian Dis **40**(3): 626-633.
http://www.ncbi.nlm.nih.gov/pubmed/8883794
Three broiler breeder houses on three different sites were sampled before and after cleansing and disinfection. None of the farms achieved total elimination of *Salmonella* Enteritidis from the poultry house environment but substantial improvements were seen when errors in the cleansing and disinfection protocol in the first house had been corrected. Fundamental errors such as over-dilution and inconsistent application of disinfectants were observed despite supervision of the process by technical advisors. In each of the three poultry units failure to eliminate a mouse population that was infected with *S.* Enteritidis was likely to be the most important hazard for the next flock.

51. Davies, R. H. and C. Wray (1997). "Distribution of *Salmonella* contamination in ten animal feedmills."
Vet Microbiol **57**(2-3): 159-169.
http://www.sciencedirect.com/science/article/pii/S0378113597001144
Detailed sampling of spillage and dust from milling equipment was carried out in nine animal feedmills, three of which were sampled twice. The *Salmonella* isolation rate ranged from 1.1% to 41.7% of the samples and the most contaminated mills were those where the inside of the cooling systems for pellet or mash had been colonised by *Salmonella*. A wide range of salmonella serotypes were isolated which included *Salmonella* Typhimurium and *S*. Enteritidis. Limited sampling every two weeks for an 18-month period in another animal feedmill showed marked variation in the contamination rate of samples and range of *Salmonella* serotypes found. Contamination of ingredient intake pits and outloading gantries for finished products by wild bird droppings containing *Salmonella* was also found in four mills.

52. Davison, S., C. E. Benson, et al. (1996). "Evaluation of disinfectants against *Salmonella* Enteritidis."
Avian Dis **40**(2): 272-277.
http://www.ncbi.nlm.nih.gov/pubmed/8790874
Five classes of disinfectants (phenol, quaternary ammonium, chlorine, glutaraldehyde, and a combination of quaternary ammonium and formaldehyde) were diluted in "field" water (well, stream, or pond water) and compared with dilutions of the disinfectants in laboratory-grade water for their efficacy against the AOAC (Association of Official Agricultural Chemists) test organism *Salmonella* Cholerasuis (ATCC 10708), *S*. Enteritidis isolated from the spleen of an infected laying hen, and an egg-invasive *S*. Enteritidis isolate. In all cases when *S*.

Cholerasuis was used, there was a significant association between the use of well, pond, and stream water and the growth of the bacterium. If we exclude glutaraldehyde, there was also a significant association between the use of "field" water and the growth of both isolates of *S.* Enteritidis. There was no significant association when glutaraldehyde was used. There was a significant association between the use of lab water and the growth of *S*. Enteritidis. The results suggested that the inability to remove *S.* Enteritidis from layer houses may in part be associated with the source of water. Variables in pH, hardness, conductivity, nitrate content, or bacterial contamination of the water did not appear to affect the ability of the disinfectant to kill *S*. Enteritidis. If "field" water is used for disinfection against *S.* Enteritidis, the use of quaternary ammonium, the combination (quaternary ammonium/formaldehyde), or phenol should be considered.

53. De Reu, K., K. Grijspeerdt, et al. (2006). "Influence of eggshell condensation on eggshell penetration and whole egg contamination with *Salmonella* enterica serovar Enteritidis." J Food Prot **69**(7): 1539-1545. http://www.ncbi.nlm.nih.gov/pubmed/16865883
Shells of agar-filled and whole eggs were inoculated with 10(3) to 10(4) CFU of *Salmonella enterica* serovar Enteritidis per eggshell. The agar-filled eggs were used to study bacterial eggshell penetration, and the whole egg results were used to characterize contamination of the egg contents. In each group, half of the eggs were stored for 21 days at 20 degrees C and 60% relative humidity (RH), and the other half was stored for 24 h at 6 degrees C and then for 20 days at 20 degrees C. The latter conditions resulted in condensation on the eggshell for 30 min from the moment the eggs were placed in the 20 degrees C chamber. Taking into account the ages at which hens were studied (39, 53, and 67 weeks), an average of 62% of the eggshells with condensate were penetrated compared with 43% for the control group; this difference was significant ($P < 0.01$). No significant difference in whole egg contamination was found; 18% of the control eggs were contaminated compared with 22% of the condensate eggs. Whole egg contamination was significantly higher for eggs from the hens at an older age (67 weeks). This difference probably was not due to a higher penetration potential because differences were not observed for the corresponding agar-filled eggs. Condensation on the eggshell seemed to encourage bacterial penetration of the eggshell but had a smaller impact on whole egg contamination.

54. de Wit, J. C. and F. M. Rombouts (1992). "Faecal micro-organisms on the hands of carriers: *Escherichia coli* as model for *Salmonella*." Zentralbl Hyg Umweltmed **193**(3): 230-236. http://www.ncbi.nlm.nih.gov/pubmed/1457034
To evaluate the public health hazard caused by *Salmonella* carriers as food handlers, a study was carried out to gather more quantitative data about faecal contamination of hands after stools. Faecal *E. coli* was used as a model bacterium for *Salmonella*. In total 92 subjects cooperated in this study. The hand was sampled before toilet use, and also after stools, with or without washing of the hands. Besides *E. coli* the number of *Enterobacteriaceae* was also determined. It appeared that hands may be contaminated with *Enterobacteriaceae*, regardless of toilet use, for a well before as after stools about 60% of the sampled hands carried a detectable number of *Enterobacteriaceae*. That is why the presence of *Enterobacteriaceae* on hands is not a good indicator for toilet hygiene. In 4% of the samples before stools and in 25% of the samples taken after stools *E. coli* could be detected (> 20 CFU/sample). The average 10 log CFU of *E. coli* in the positive samples taken before and after stools were about the same: 2.30 per sample. Hand washing after stools reduced the numbers of *Enterobacteriaceae* and *E. coli* on the hands. It was concluded that symptomless *Salmonella* excretors in the period starting two weeks after infection form only a low risk in carrying over *Salmonella* by their hands to food. Especially if normal hygiene is practiced like washing hands after stools, the number of contaminated hands will be very low and furthermore the number of faecal micro-organisms will be very small.

55. Denehy, E. J., J. C. Raupach, et al. (2011). "Outbreak of *Salmonella* Typhimurium phage type 44 infection among attendees of a wedding reception, April 2009." Commun Dis Intell Q Rep **35**(2): 192-196. http://www.ncbi.nlm.nih.gov/pubmed/22010514

On 30 April 2009, the Communicable Disease Control Branch (CDCB) South Australia was notified of a *Salmonella* infection in a person who attended a wedding reception on 25 April 2009. Several other attendees reported becoming unwell with a similar gastrointestinal illness. The CDCB commenced an investigation to: characterise the outbreak in terms of person, place and time; identify probable source or sources; and implement control measures. A retrospective cohort study was undertaken among wedding reception attendees. A questionnaire collecting information on demographics, illness and menu items consumed was given to the majority of attendees. An environmental inspection of the wedding reception premise and food supplier premise, including food sampling was conducted to identify plausible sources of infection. The questionnaire response rate was 77%, from which an attack rate of 20% was calculated. There was a significant association between consumption of garlic aioli and illness (OR 5.4, 95% CI: 1.6, 18.1). Nine wedding reception attendees' stool samples tested positive for *Salmonella* Typhimurium phage type 44. A sample of garlic aioli also tested positive for *Salmonella* Typhimurium phage type 44. The ingredients of the garlic aioli included raw egg yolk, roasted garlic, Dijon mustard, vinegar and vegetable oil. The raw egg yolk was identified as a high risk food item; however no eggs tested positive for *Salmonella*.

56. Denys, S., J. Pieters, et al. (2007). CFD Analysis of thermal processing of eggs. Computational fluid dynamics in food processing. D.-W. Sun. Boca Raton, FL, Taylor & Francis Group.

57. Desin, T. S., W. Koster, et al. (2013). "*Salmonella* vaccines in poultry: past, present and future." Expert Rev Vaccines **12**(1): 87-96. http://www.ncbi.nlm.nih.gov/pubmed/23256741
Salmonella species are important zoonotic pathogens that cause gastrointestinal disease in humans and animals. Poultry products contaminated with these pathogens are one of the major sources of human *Salmonella* infections. Vaccination of chickens, along with other intervention measures, is an important strategy that is currently being used to reduce the levels of *Salmonella* in poultry flocks, which will ultimately lead to lower rates of human *Salmonella* infections. However, despite numerous studies that have been performed, there is still a need for safer, well-defined *Salmonella* vaccines. This review examines the different classes of *Salmonella* vaccines that have been tested, highlighting the merits and problems of each, and provides an insight into the future of *Salmonella* vaccines and the platforms that can be used for delivery.

58. Devi, S. J. and C. J. Murray (1991). "Cockroaches (*Blatta* and *Periplaneta* species) as reservoirs of drug-resistant salmonellas." Epidemiol Infect **107**(2): 357-361. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2272068/pdf/epidinfect00029-0117.pdf
A total of 221 cockroaches (*Blatta* and *Periplaneta* spp.), collected in hospitals, houses, animal sheds, grocery stores and restaurants, in various parts of South Kanara District, a south-west coastal region of India, were studied bacteriologically for the presence of various salmonellas. Salmonellas were isolated from 4.1% of these cockroaches. Nine strains of salmonellas were recovered, belonging to five serotypes--*Salmonella* Bovismorbificans, *S.* Oslo, *S.* Typhimurium, *S.* Mbandaka and *S.* Braenderup, the former two being the commonest serotypes. All salmonellas were resistant to one or other of 11 antibacterial drugs used in the susceptibility test. Isolation of salmonellas from cockroaches collected from the livestock premises and human dwellings suggested that they may act as significant reservoirs of *Salmonella* in nature. Recovery of serotypes, phage types and R-types that were commonly isolated from humans and animals of this locality, suggested a transmission role for cockroaches. By harbouring potentially pathogenic, drug-resistant salmonellas, these wandering arthropods may pose dangerous infective hazards to humans and animals.

59. Doyle, M. E. and A. S. Mazzotta (2000). "Review of studies on the thermal resistance of Salmonellae." J Food Prot **63**(6): 779-795. http://www.ncbi.nlm.nih.gov/pubmed/10852574
Heat resistance data for different serotypes of *Salmonella* enterica in different food products and laboratory media are reviewed. From all D-values reported, the highest heat resistance of

Salmonella was in liquid eggs and liquid egg yolks. The equation from a line drawn through the highest D-values, and above all values reported, was log D-value = 11.7 - 0.188T degrees C. From this equation, the calculated z-value was 5.3 degrees C (9.5 degrees F), and a process at 71degrees C (160 degrees F) will require 1.2 s to inactivate 1 log of *Salmonella* cells. This calculation did not include data that evaluated the heat resistance after stress conditions or data for *Salmonella* Senftenberg. The heat resistance of *Salmonella* is highly influenced by the strain tested, the type of experiment (log reduction versus end-point), culture conditions prior to the experiment, heating menstruum, and recovery conditions. Heat resistance data for *Salmonella* are still nonexistent or scarce in chicken meat, fruit juices, and aquacultured fish.

60. Dryden, M. S., N. Keyworth, et al. (1994). "Asymptomatic foodhandlers as the source of nosocomial salmonellosis."
J Hosp Infect **28**(3): 195-208.
http://www.sciencedirect.com/science/article/pii/0195670194901023
A nosocomial outbreak of salmonellosis affected 22 patients and seven staff on 14 wards in two hospitals with shared catering facilities. The outbreak was characterized by a low level intermittent infection with *Salmonella* Enteritidis phage type 4 over a 19-day period. The epidemiology did not suggest a common source for the outbreak and there was little evidence for person-to-person spread. Extensive food and environmental sampling failed to yield *Salmonella*. Control measures within the kitchens and on wards early in the outbreak had no effect on the rate of infection. Faecal screening of asymptomatic people demonstrated a high carriage rate among catering staff (12·3%), compared with ward staff (2·2%) or patients (0·8%). A case-control study failed to reveal any association between illness and particular meals, food types, wards, medical attendants, medical procedures, or medication. However there was an association between illness and eating meals prepared by one carrier (P = 0·02). Transmission was believed to be via intermittent contamination of occasional meals. No further cases occurred after the exclusion of infected food handlers. The identifiable costs of the outbreak amounted to approximately £33 000. These results indicate that asymptomatic food handlers may be the source of nosocomial salmonella outbreaks, and that efforts should be made to identify carriers and treat them.

61. Dunowska, M., P. S. Morley, et al. (2005). "The effect of Virkon S fogging on survival of *Salmonella* enterica and *Staphylococcus aureus* on surfaces in a veterinary teaching hospital."
Vet Microbiol **105**(3-4): 281-289.
http://www.biosicurezzaweb.net/pdf/VirkonS/04.pdf
The objective of the study was to determine the disinfection efficacy of aerosolizing (cold fogging) Virkon S on survival of *Stahpylococcus aureus* and *Salmonella* enterica on different surfaces. Two experiments were conducted in different locations. *Salmonella* enterica and S. aureus were grown in broth culture and then seeded into pre-marked areas in each location and allowed to dry. Virkon S (1%) was aerosolized into the rooms (approximately 1L of per 30 m(3)). Samples were collected pre- and post-fogging for quantitative cultures to evaluate the efficacy of aerial disinfection. The reduction of *S.* enterica or *S. aureus* counts ranged from 3.40 to 0.95 log(10) (Salmonella) or 4.92 to 0.02 log(10) (*Staphylococcus*). The greatest reduction was evident in samples collected from non-porous horizontal surfaces, which were not obstructed from the air flow. These results indicate that fogging with Virkon S could be beneficial in routine disinfection of pre-cleaned surfaces. The benefits of routine use of cold fogging with Virkon S in veterinary hospital settings would include its wide-range antimicrobial action and minimal working-men power required to disinfect large areas. Also, fogging would potentially minimize microbial contamination in the hard to reach areas.

62. Ebel, E. D., J. Mason, et al. (1993). "Occurrence of *Salmonella* Enteritidis in unpasteurized liquid egg in the United States."
Avian Dis **37**(1): 135-142.
http://www.ncbi.nlm.nih.gov/pubmed/8452489
In order to gain a greater understanding of the occurrence and distribution of *Salmonella* Enteritidis in the United States, a survey of unpasteurized liquid egg (collected at 20 egg-breaking plants across the United States) was conducted over a 52-week period. Weekly liquid egg samples were submitted for *Salmonella* culturing at the National Veterinary

Services Laboratories. Group D positive salmonellae were serotyped and phage-typed. On a regional basis, the Northern Region of the United States had the highest *S*. Enteritidis recovery, with 20% of the samples submitted from plants in that region culture-positive for this serotype. *Salmonella* Enteritidis positives from the Southeast, Central, and Western regions were 10%, 15%, and 6% of the samples submitted, respectively.

63. EFSA, P. o. B. H. (2005). "Microbiological risks on washing of table eggs." EFSA Journal **269**:1-39 http://www.efsa.europa.eu/en/scdocs/doc/269.pdf
Concerning marketing standards of eggs, Regulation no. 2295/2003, defines 2 grades of eggs (A and B) according to different physical characteristics as follows: (i) Grade A eggs ("fresh eggs" or "table eggs") should have a "normal, clean and undamaged" shell and cuticle; they will not be washed or cleaned before or after grading, and will be not chilled or treated for preservation."; (ii) Grade B eggs, i.e. eggs "which do not meet requirements applicable to eggs in grade A", may only be used by the food or non-food industries. It is important to produce eggs that present the lowest health risks for the consumer. A major challenge is that shell eggs are considered a primary source of human salmonellosis in Europe.

64. EFSA, P. o. B. H. (2009). "Special measures to reduce the risk for consumers through *Salmonella* in table eggs – e.g. cooling of table eggs." EFSA Journal **957**: 1-29. http://www.efsa.europa.eu/en/scdocs/doc/957.pdf
Following a request from the German Federal Institute for Risk Assessment the Scientific Panel on Biological Hazards was asked to deliver a scientific opinion on special measures to reduce the risk for consumers through *Salmonella* in table eggs, e.g. cooling of eggs. As stated in the EFSA Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in 2007, the reported number of cases and incidence of human salmonellosis in the EU were, respectively, 154,099 cases and 31.1 cases per 100,000 inhabitants. The report also documents that the *Salmonella* prevalence in table eggs was 0.8%. According to an opinion from the Scientific Committee on Veterinary Measures relating to Public Health on Salmonellae in Foodstuffs (2003), eggs and products containing raw eggs are among the food categories most likely to pose the greatest risk to public health in relation to salmonellosis. Table eggs are identified as a major source of *Salmonella*, and egg refrigeration has been suggested as one of many possible interventions along the food chain to reduce the incidence of salmonellosis in the human population. On the other hand, problems associated with this measure have long since been highlighted, including those resulting from an inability to maintain the cold chain and the consequential water condensation on the egg surface which facilitates growth and penetration of microorganisms into the egg. Additionally, rapid cooling may provoke cracks in eggs because of temperature gradients and this may further facilitate microbial migration through the shell.

65. El-Lethey, H., B. Huber-Eicher, et al. (2003). "Exploration of stress-induced immunosuppression in chickens reveals both stress-resistant and stress-susceptible antigen responses." Vet Immunol Immunopathol **95**(3-4): 91-101. http://www.ncbi.nlm.nih.gov/pubmed/12963270
In the present study, depriving chickens of foraging material was shown to induce stress. The impact of this type of stress on the immune response was compared with feeding of corticosterone (1.5 mg per bird per day), a hormone known to be immunosuppressive and to be the major stress hormone of chickens. Corticosterone feeding induced stress as revealed by higher heterophil/lymphocyte (H/L) ratios, longer tonic immobility (TI) reaction, reduced body weight gain and reduced egg production. Blood corticosterone levels were increased. Corticosterone feeding decreased the antibody response to tetanus toxoid and SRBC, DTH to PPD from Mycobacterium tuberculosis and the inflammatory response to PHA. Housing chickens on slats also induced chronic stress, as evidenced by increased H/L ratios, prolonged TI duration and decreased egg production. Corticosterone levels were slightly but not significantly enhanced. This novel form of chronic stress strongly suppressed humoral and cellular immune responses as evidenced by lower antibody titers to sheep red blood cells (SRBC) and tetanus toxoid (TT) decreased DTH reaction to PPD and inflammatory reaction to PHA in the skin. In contrast, the antibody response to human serum albumin (HSA) was

neither influenced by corticosterone feeding nor by keeping the birds on slats. Even the combination of corticosterone feeding and housing the birds on slats did not significantly impair antibody responses to HSA. In conclusion, the present study showed that chronic stress induced by depriving the birds of foraging material led to a similar impairment of humoral and cell-mediated immunity as did feeding with corticosterone. More importantly, it showed for the first time that depending on the antigen tested, there are stress-resistant and stress-susceptible antigen responses.

66. Er, B., B. Demirhan, et al. (2014). "Antimicrobial and antibiofilm effects of selected food preservatives against *Salmonella* spp. isolated from chicken samples."
Poult Sci **93**(3): 695-701.
http://ps.oxfordjournals.org/content/93/3/695.long
Salmonella spp. are widespread foodborne pathogens that contaminate egg and poultry meats. Attachment, colonization, as well as biofilm formation capacity of *Salmonella* spp. on food and contact surfaces of food may cause continuous contamination. Biofilm may play a crucial role in the survival of salmonellae under unfavorable environmental conditions, such as in animal slaughterhouses and processing plants. This could serve as a reservoir compromising food safety and human health. Addition of antimicrobial preservatives extends shelf lives of food products, but even when products are supplemented with adequate amounts of preservatives, it is not always possible to inhibit the microorganisms in a biofilm community. In this study, our aims were i) to determine the minimum inhibitory concentrations (MIC) and minimum biofilm inhibitory concentrations (MBIC) of selected preservatives against planktonic and biofilm forms of *Salmonella* spp. isolated from chicken samples and *Salmonella* Typhimurium SL1344 standard strain, ii) to show the differences in the susceptibility patterns of same strains versus the planktonic and biofilm forms to the same preservative agent, and iii) to determine and compare antimicrobial and antibiofilm effects of selected food preservatives against *Salmonella* spp. For this purpose, *Salmonella* Typhimurium SL1344 standard strain and 4 *Salmonella* spp. strains isolated from chicken samples were used. Investigation of antimicrobial and antibiofilm effects of selected food preservatives against *Salmonella* spp. was done according to Clinical and Laboratory Standards Institute M100-S18 guidelines and BioTimer assay, respectively. As preservative agents, pure ciprofloxacin, sodium nitrite, potassium sorbate, sodium benzoate, methyl paraben, and propyl paraben were selected. As a result, it was determined that MBIC values are greater than the MIC values of the preservatives. This result verified the resistance seen in a biofilm community to food preservatives and highlighted this subject, not to be ignored in food applications.

67. FDA, F. a. D. A. (2009). "Prevention of *Salmonella* Enteritidis in shell eggs during production, storage, and transportation. Final rule."
Fed Regist **74**(130): 33029-33101.
http://www.ncbi.nlm.nih.gov/pubmed/19588581
The Food and Drug Administration (FDA) is issuing a final rule that requires shell egg producers to implement measures to prevent *Salmonella* Enteritidis (SE) from contaminating eggs on the farm and from further growth during storage and transportation, and requires these producers to maintain records concerning their compliance with the rule and to register with FDA. FDA is taking this action because SE is among the leading bacterial causes of foodborne illness in the United States, and shell eggs are a primary source of human SE infections. The final rule will reduce SE-associated illnesses and deaths by reducing the risk that shell eggs are contaminated with SE.

68. Fedorka-Cray, P. J., A. Hogg, et al. (1997). "Feed and feed trucks as sources of *Salmonella* contamination in swine."
Swine Health Prod **5**(5): 189-193.
https://www.aasv.org/shap/issues/v5n5/v5n5p189.pdf
Purpose: To investigate whether feed trucks and feed could be sources of *Salmonella* contamination in swine operations.
Materials: Five hundred forty-nine swab samples were taken from approximately 25 different places in the grain box on 22 different feed trucks and cultured for *Salmonella* spp. In addition, a sample of the feed components from 17 of those trucks was cultured for the presence of *Salmonella*.

Results: Culture of both truck swab and feed samples indicate that *Salmonella* was present on five of the 22 (22.7%) trucks. A total of 549 swabs were cultured and the rate of isolation was 0.7% (four of 549 positive for *Salmonella*). Positive swabs were recovered from three trucks for a recovery rate of 13.6%. Feed was submitted for 17 of 22 trucks and *Salmonella* was recovered from four of 17 samples (23.5%). However, positive swabs and feed samples only matched for two trucks. No trucks had been used to transport livestock within the past 30 days nor were any trucks cleaned or disinfected between loads. Implications: While sample prevalence of *Salmonella* in feed trucks is low (0.7%), the overall contamination rate for feed trucks is much higher (22.7%). The presence of positive feed samples suggests that feed could be a source of *Salmonella* contamination for swine.

69. Foley, S. L., R. Nayak, et al. (2011). "Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production."
Appl Environ Microbiol **77**(13): 4273-4279.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3127710/
Fresh and processed poultry have been frequently implicated in cases of human salmonellosis. Furthermore, increased consumption of meat and poultry has increased the potential for exposure to *Salmonella enterica*. While advances have been made in reducing the prevalence and frequency of *Salmonella* contamination in processed poultry, there is mounting pressure on commercial growers to prevent and/or eliminate these human pathogens in preharvest production facilities. Several factors contribute to *Salmonella* colonization in commercial poultry, including the serovar and the infectious dose. In the early 1900s, *Salmonella* enterica serovars Pullorum and Gallinarum caused widespread diseases in poultry, but vaccination and other voluntary programs helped eradicate pullorum disease and fowl typhoid from commercial flocks. However, the niche created by the eradication of these serovars was likely filled by *S.* Enteritidis, which proliferated in the bird populations. While this pathogen remains a significant problem in commercial egg and poultry production, its prevalence among poultry has been declining since the 1990s. Coinciding with the decrease of *S.* Enteritidis, *S.* Heidelberg and *S.* Kentucky have emerged as the predominant serovars in commercial broilers. In this review, we have highlighted bacterial genetic and host-related factors that may contribute to such shifts in *Salmonella* populations in commercial poultry and intervention strategies that could limit their colonization.

70. Garber, L., M. Smeltzer, et al. (2003). "*Salmonella enterica* serotype Enteritidis in table egg layer house environments and in mice in U.S. layer houses and associated risk factors."
Avian Dis **47**(1): 134-142.
http://www.ncbi.nlm.nih.gov/pubmed/12713168
Prevalence was estimated for *Salmonella enterica* serotype eneritidis (SE) in layer house environments (n = 200 layer houses) and house mice (n = 129 layer houses) in 15 states throughout the United States. Environmental swabs were collected from manure, egg belts, elevators, and walkways. Live-catch rodent traps were placed for 4-7 days. Swabs and house mice were submitted to the laboratory for bacterial culture. Overall, 7.1% of layer houses and 3.7% of mice were culture positive for SE. The highest prevalence was in the Great Lakes region of the United States, and no SE was recovered from houses or mice in the southeast region. Presence of SE in layer houses was associated with age/molting, floor reared pullets, and number of rodents trapped. Cleaning and disinfecting houses between flocks was associated with a reduced risk. The prevalence of SE in mice from environmentally positive houses was nearly four times that of mice from environmentally negative houses.

71. Gast, R. K. and R. Guraya (2013). "Continuing multiplication of *Salmonella* Enteritidis strains in egg yolk during refrigeration at 7.2^{o}C."
Int J Poult Sci **12**(11): 622-627
http://www.pjbs.org/ijps/fin2492.pdf
The continuing attribution of human illness caused by *Salmonella* Enteritidis to the consumption of contaminated eggs has led to widespread implementation of risk reduction programs for commercial egg production, often emphasizing prompt refrigeration of eggs to prevent bacterial multiplication to dangerously high levels. However, microbial growth may not cease immediately inside warm eggs after transfer to refrigerated storage. The present study compared the abilities of 8 *S.* Enteritidis strains (of 4 phage types) to continue multiplying in experimentally contaminated egg yolk during the first 24 h after transition from warm to

refrigeration temperatures. After 15 mL samples of egg yolk were inoculated with 10 CFU/ml of *S.* Enteritidis, they were incubated at 37°C for 16 h and then transferred into refrigeration at 7.2°C for 24 h. Bacterial cell concentrations were determined following 37°C incubation and again after both 8 and 24 h at 7.2°C. All 8 *S.* Enteritidis isolates multiplied significantly during 16 h of incubation, reaching an overall mean of log10 8.790 CFU/ml. After refrigeration, the observed mean values for cell concentrations in yolk samples were log10 8.780 CFU/mL at 8 h and log10 8.849 CFU/mL at 24 h. For 3 of 8 strains, a significant ($p<0.05$) increase in cell concentrations in egg yolk occurred during 24 h of refrigeration. These results support the importance of prompt egg refrigeration for minimizing the numbers of *S.* Enteritidis in marketed table eggs, although refrigeration at 7.2°C may not immediately or completely arrest multiplication by all strains.

72. Gast, R. K., R. Guraya, et al. (2007). "In vitro penetration of egg yolks by *Salmonella* Enteritidis and *Salmonella* Heidelberg strains during thirty-six-hour ambient temperature storage."
Poult Sci **86**(7): 1431-1435.
http://ps.oxfordjournals.org/content/86/7/1431.abstract
Although *Salmonella* deposition inside yolks is uncommon in naturally contaminated eggs, migration through the vitelline membrane into the nutrient-rich yolk contents could enable rapid bacterial multiplication. Egg refrigeration restricts both penetration and growth, but a recently proposed national *Salmonella* Enteritidis control program would allow unrefrigerated ambient temperature storage of eggs on farms for up to 36 h. The present study used an in vitro egg contamination model to assess the ability of small numbers of 4 *Salmonella* Enteritidis strains and 4 *Salmonella* Heidelberg strains to penetrate the vitelline membrane and multiply inside yolks during 36 h of storage at either 20 or 30°C. After inoculation onto the exterior surface of the vitelline membrane, all 8 *Salmonella* strains penetrated to the yolk contents (at a mean frequency of 45.1%), and most strains grew to significantly higher levels (with a mean log10 bacterial concentration of 2.2 cfu/mL) during incubation at 30°C. Significant differences in penetration frequency and yolk multiplication were observed between individual strains and between serotypes (*Salmonella* Enteritidis > *Salmonella* Heidelberg for both parameters). Penetration and multiplication were significantly less frequent during incubation at 20°C. These results demonstrate that controlling ambient temperatures during prerefrigeration storage may be an important adjunct to prompt refrigeration for limiting *Salmonella* growth in eggs and thereby for preventing egg-transmitted human illness.

73. Gast, R. K. and P. S. Holt (2001). "Assessing the frequency and consequences of *Salmonella* Enteritidis deposition on the egg yolk membrane."
Poult Sci **80**(7): 997-1002.
http://naldc.nal.usda.gov/download/23659/PDF
The site of deposition of *Salmonella* Enteritidis in eggs could influence the extent to which this pathogen multiplies before refrigeration achieves growth-inhibiting internal temperatures. The first part of this study sought to determine whether *S.* Enteritidis inoculated onto the exterior (vitelline) membrane surface of egg yolks was able to penetrate into and multiply within the yolk contents. When 10(2) cfu of *S.* Enteritidis was inoculated onto the exterior surface of intact egg yolks, multiplication within the interior yolk contents occurred in 10% of samples after 6 h of incubation and in 75% of samples after 24 h at 25 C (reaching mean levels of about 10(4) cfu/mL) but in only 20% of samples incubated for 72 h at 15 C. The second part of this study applied an oral infection model in laying hens to establish the relative proportions of contaminated eggs in which *S.* Enteritidis deposition was associated with the yolk membrane or was found inside the yolk contents. Although approximately 4.3% of egg yolks were positive for *S.* Enteritidis when both yolk contents and membranes were sampled, only about 0.5% of samples of yolk contents (without membranes) were positive. Although deposition of S. enteritidis within egg yolks appears to occur infrequently, rapid refrigeration of eggs is necessary to prevent the penetration of *S.* Enteritidis into and multiplication within egg yolks.

74. Gast, R. K. and P. S. Holt (2001). "Multiplication in egg yolk and survival in egg albumen of *Salmonella enterica* serotype Enteritidis strains of phage types 4, 8, 13a, and 14b."
J Food Prot **64**(6): 865-868.

http://www.ncbi.nlm.nih.gov/pubmed/11403140
Refrigeration of eggs is vital for restricting the multiplication of *Salmonella enterica* serotype Enteritidis contaminants, but differences between *Salmonella* Enteritidis strains or phage types in their survival and multiplication patterns in egg contents might influence the effectiveness of refrigeration standards. The present study compared the abilities of 12 *Salmonella* Enteritidis isolates of four phage types (4, 8, 13a, and 14b) to multiply rapidly in egg yolk and to survive for several days in egg albumen. The multiplication of very small numbers of *Salmonella* Enteritidis inoculated into yolk (approximately 10(1) CFU/ml) was monitored during 24 h of incubation at 25 degrees C, and the survival of much larger numbers of *Salmonella* Enteritidis inoculated into albumen (approximately 10(5) CFU/ml) was similarly evaluated during the first 3 days of incubation at the same temperature. In yolk, the inoculated *Salmonella* Enteritidis strains multiplied to mean levels of approximately 10(3) CFU/ml after 6 h of incubation and 10(8) CFU/ml after 24 h. In albumen, mean levels of approximately 10(4) CFU/ml or more of *Salmonella* Enteritidis were maintained through 72 h. Although a few differences in multiplication and survival were observed between individual isolates, the overall range of values was relatively narrow, and no significant differences ($P < 0.05$) were evident among phage types.

75. Gast, R. K., D. R. Jones, et al. (2010). "In vitro penetration of *Salmonella* Enteritidis through yolk membranes of eggs from 6 genetically distinct commercial lines of laying hens." Poult Sci **89**(8): 1732-1736.
http://ps.oxfordjournals.org/content/89/8/1732.long
Although deposition of *Salmonella* Enteritidis inside yolks is less common than deposition in albumen or on the vitelline (yolk) membrane in naturally contaminated eggs laid by infected hens, bacterial migration into the yolk to reach its nutrient-rich contents could lead to extensive multiplication. The present study used an in vitro egg contamination model to assess the ability of small initial numbers of *Salmonella* Enteritidis to penetrate the vitelline membrane and multiply inside yolks of eggs laid by 6 genetically distinct commercial lines of hens during 24 h of storage at 30 degrees C. Eggs from each line were tested at 4 different hen ages by inoculation of approximately 100 cfu of *Salmonella* Enteritidis onto the outside of the vitelline membranes of intact yolks in plastic centrifuge tubes and then adding back the albumen into each tube before incubation. Overall, the frequency of penetration of *Salmonella* Enteritidis into the yolk contents of eggs from individual lines of hens ranged from 30 to 58% and the mean concentration of *Salmonella* Enteritidis in yolk contents after incubation ranged from 0.8 to 2.0 log(10) cfu/mL. For both of these parameters, values for one hen line were significantly higher than for 2 other lines, but no other differences were observed. Hen age did not have a significant effect on egg yolk penetration by *Salmonella* Enteritidis. These results indicate that opportunities for the migration and growth of small initial numbers of *Salmonella* Enteritidis to attain more dangerous levels inside contaminated eggs during storage at warm temperatures can sometimes vary between different lines of laying hens.

76. Gerba, C. P., A. H. Tamimi, et al. (2014). "Bacterial occurrence in kitchen hand towels." Food Prot Trends **34**(5): 312-317.
http://www.foodprotection.org/files/food-protection-trends/Sep-Oct-14-Gerba.pdf
The common occurrence of enteric bacteria in kitchen sponges and dishcloths suggests that they can play a role in the cross-contamination of foods, fomites and hands by foodborne pathogens. This study investigated the occurrence of bacteria in kitchen towels often used to dry dishes, hands and other surfaces in the domestic kitchen. A total of 82 kitchen hand towels were collected from households in five major cities in the United States and Canada and the numbers of heterotrophic bacteria, coliform bacteria, and *Escherichia coli* in each towel were determined. In addition, identification of the enteric bacteria was performed on selected towels. Coliform bacteria were detected in 89.0% and E. coli in 25.6% of towels. The presence of *E. coli* was related to the frequency of washing.

77. Gole, V. C., K. K. Chousalkar, et al. (2014). "Effect of egg washing and correlation between eggshell characteristics and egg penetration by various *Salmonella* Typhimurium strains." PLoS One **9**(3): e90987.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3951326/pdf/pone.0090987.pdf
Salmonella is an important foodborne pathogen, causing an estimated 11,992 cases of infection in Australia per year. Egg or egg product related salmonellosis is a major concern for

the egg industry. Worldwide, *S.* Typhimurium is one of the most common serovars identified in *Salmonella* food poisoning cases. The current study investigated the ability of five *S.* Typhimurium strains to penetrate washed and unwashed eggs using whole egg and agar egg penetration methods. All *S.* Typhimurium strains were able to penetrate eggshells and survive in egg albumen (at 20 degrees C) according to whole egg penetration results. Polymerase Chain Reaction results demonstrated that *S.* Typhimurium strain 2 (10(3) and 10(5) CFU/mL), and strain 5 (10(3) and 10(5) CFU/mL) egg penetration was significantly higher ($p<0.05$) in washed eggs when compared to unwashed eggs. Statistical analysis of the agar penetration experiment indicated that *S.* Typhimurium was able to penetrate washed eggs at a significantly higher rate when compared to unwashed eggs ($p<0.05$). When compared to unwashed eggs, washed eggs also had significantly damaged cuticles. Statistical analysis also indicated that eggshell penetration by *S.* Typhimurium was related to various eggshell ultrastructural features such as cap quality, alignment, erosion, confluence, Type B bodies and cuticle cover.

78. Gole, V. C., V. Torok, et al. (2014). "Association between the indoor environmental contamination of *Salmonella* with egg contamination on layer farms."
J Clin Microbiol. **52**(9): 3250-3258
http://jcm.asm.org/content/52/9/3250.long
The current study involved longitudinal and point in time surveys of *Salmonella* carriage and environmental contamination on two commercial cage layer farms positive for *Salmonella* Typhimurium (flock A age = 32 weeks; and flock B age = 34 weeks). *Salmonella* positive faecal samples, egg belt, and dust were all unconditionally associated with eggshells testing positive for *Salmonella*. The odds of an eggshell testing positive for *Salmonella* were 91.8, 61.5 and 18.2 times higher when faecal, egg belt and dust samples tested *Salmonella* positive. Agreement between culture based methods and real time polymerase chain reaction on pre-enriched broths in detecting *Salmonella* was almost perfect for eggshell (observed agreement=99.19%, Kappa coefficient=0.94) and egg belt samples (observed agreement= 95%, Kappa coefficient=0.88), and substantial for faecal (observed agreement=87.14%, Kappa coefficient=0.47) and floor dust samples (observed agreement=80.61%, kappa coefficient=0.58). One log increase in the load of *Salmonella* detected in faecal, egg belt and floor dust samples resulted in 35%, 43% and 45% increase ($p<0.001$) in the odds of an eggshell testing positive for *Salmonella* respectively. Multi-locus variable number tandem repeat analysis (MLVA) of *S.* Typhimurium strains isolated from flock A were distinct from flock B. *S.* Typhimurium strains detected from human food poisoning cases exhibited similar MLVA pattern to the strains isolated from flock A and B.

79. Gormley, F. J., C. L. Little, et al. (2010). "Pooling raw shell eggs: *Salmonella* contamination and high risk practices in the United Kingdom food service sector."
J Food Prot **73**(3): 574-578.
http://www.ncbi.nlm.nih.gov/pubmed/20202348
Salmonella contamination of pooled raw shelled egg mix (RSEM) used as an ingredient in lightly cooked or uncooked foods and high-risk kitchen hygiene practices in United Kingdom food service establishments using RSEM were investigated. Samples were collected from 934 premises. *Salmonella* was found in 1 (0.13%) of 764 RSEM samples, 2 (0.3%) of 726 samples from surfaces where ready-to-eat foods were prepared, and 7 (1.3%) of 550 cleaning cloths. Poor RSEM storage and handling practices were highlighted. Workers in 40% of the premises sampled failed to use designated utensils when RSEM was added to other ingredients, workers in 17% of the premises did not clean surfaces and utensils thoroughly after use with RSEM and before preparing other foods, only 42% of workers washed and dried their hands after handling eggs or RSEM, workers in 41% of the premises did not store RSEM at refrigeration temperature before use, and workers in 8% of the premises added RSEM to cooked rice at the end of cooking when preparing egg fried rice. Take-away premises, especially those serving Chinese cuisine, were least likely to have a documented food safety management system and awareness of the key food safety points concerning the use of RSEM compared with other food service premises ($P < 0.0001$). Food service businesses using RSEM must be aware of the continuing hazard from *Salmonella*, must adopt appropriate control measures, and must follow advice provided by national food agencies to reduce the risk of *Salmonella* infection.

80. Greenberg, B. and M. Klowden (1972). "Enteric bacterial interactions in insects."
Am J Clin Nutr **25**(12): 1459-1466.
http://ajcn.nutrition.org/content/25/12/1459.abstract
http://ajcn.nutrition.org/content/25/12/1459.full.pdf
The potential of maggots and adult flies to harbor and disseminate *Salmonella* and *Shigella* was investigated gnotobiotically after it was found that a majority of larvae and all of the emerging adults breeding in normally contaminated media plus *Salmonella* Typhi or *Shigella flexneri* failed to retain either pathogen. When flies were raised in the absence of other microorganisms, *Shigella flexneri*, *Salmonella* Typhi, *S.* Paratyphi B., *S*. Typhimurium, and *S*. Enteritidis, although reduced in numbers, survived pupation. In vivo bicontaminant interactions of *S.* Typhimurium with *E. coli*, *Streptococcus faecalis*, and *Proteus mirabilis* identified *P. mirabilis* as the major antagonist of *Salmonella*. Although equal numbers of both organisms were introduced initially, *Proteus*: *Salmonella* ratios reached 11,500:1 in the maggot gut, compared with a maximum of 20:1 in a nutrient broth culture. In searching for an in vitro model that would parallel the in vivo antibacterial effects, a filtrate of a 15-day broth culture of *Proteus mirabilis* at pH 2.5 to 3.0 was found to demonstrate the extremely rapid kill and acid-dependent activity occurring in the gut of the maggot.In the gnotobiotic adult house fly, inputs of 22 *S*. Typhimurium produced excretion of the pathogen in 27 out of 45 flies, with outputs as high as 1.4 x 107. As input increased, the percentage of *Salmonella* excreters generally also increased until all flies showed bacteria in their defecations at inputs of 1.3 x 104. The green bottle fly appeared to be a poorer host for *Salmonella*, with lower percentages of bacterial excreters and multipliers observed. Antagonisms of *Salmonella* by *Proteus* were again evident in the adult. The excretion of *Salmonella* was reduced to 1 day, and the percentage of flies showing excretion on that day was 27%, compared with 8 days of excretion and 87% on the 1st day in the case of monocontaminated flies. A mouse fecal flora substituted for *Proteus* exerted a lesser degree of antagonism to *Salmonella*.Overall data show the adult fly has the most potential for disease transmission, as the maggot has limited motility, and possesses autosterilization mechanisms which limit its capacity to carry pathogens over into the adult stage.

81. Greig, J. D. and A. Ravel (2009). "Analysis of foodborne outbreak data reported internationally for source attribution."
Int J Food Microbiol **130**(2): 77-87.
http://www.ncbi.nlm.nih.gov/pubmed/19178974
Analysis of foodborne outbreak data is one approach to estimate the proportion of human cases of specific enteric diseases attributable to a specific food item (food attribution). Although we recognize that for a variety of reasons reported outbreaks represent only a small portion of all actual outbreaks, using outbreak data for food attribution is the only methodological approach where, theoretically, there is an actual direct link between the pathogen, its source and each infected person. The purpose of this study was to explore the usefulness of foodborne outbreak data extracted from publicly available international electronic reports and publications to provide estimates of food attribution, to derive and compare these estimates between regions, while improving the understanding of the pathogen/food vehicle combination. Electronic reports and publications of foodborne outbreaks that occurred globally since the 1980s were systematically scanned and their data were extracted and compiled in a database. A system of food categorization was developed and food vehicles assigned accordingly. The association between the aetiology and the food source was statistically described for outbreaks with both reported aetiology and incriminated food vehicle. Differences in associations between Australia and New Zealand, Canada, the European Union (EU) and the United States (US) were explored using multiple correspondence analysis and were formally tested between the EU and the US for selected pathogens and food sources. As a result, the food and aetiology cross tabulation of 4093 foodborne outbreaks that occurred globally between 1988 and 2007 is presented and discussed. For a few aetiologies and some foods the association is very specific. The lack of a specific association between the other foods and aetiologies highlights the potential roles of cross-contamination, environmental contamination and the role of the infected foodhandler along the food chain from farm to fork. Detailed analysis of the four regions highlighted some specific associations: *Salmonella* Enteritidis outbreaks occurred relatively often in the EU states with eggs as the most common source; *Campylobacter* associated outbreaks were mainly related to poultry products in the EU and to dairy products in the US; there was an

association between *Escherichia coli* outbreaks and beef in Canada; and while *Salmonella* Typhiumurium outbreaks were relatively common in Australia and New Zealand, across all regions, *Salmonella* was associated with a variety of food groups. The value and limitations of the study are discussed, as well as the extrapolation of the food attribution estimates beyond their outbreak context.

82. Greig, J. D., E. C. D. Todd, et al. (2007). "Outbreaks where food workers have been implicated in the spread of foodborne disease. part 1. Description of the problem, methods, and agents involved."
J Food Prot **70**(7): 1752-1761.
http://www.ingentaconnect.com/content/iafp/jfp/2007/00000070/00000007/art00031
Food workers in many settings have been responsible for foodborne disease outbreaks for decades, and there is no indication that this is diminishing. The Committee on Control of Foodborne Illnesses of the International Association for Food Protection was tasked with collecting and evaluating any data on worker-associated outbreaks. A total of 816 reports with 80,682 cases were collected from events that occurred from 1927 until the first quarter of 2006. Most of the outbreaks reviewed were from the United States, Canada, Europe, and Australia, with relatively few from other parts of the world, indicating the skewed set of data because of availability in the literature or personal contact. Outbreaks were caused by 14 agents: norovirus or probable norovirus (338), *Salmonella enterica* (151), hepatitis A virus (84), *Staphylococcus aureus>* (53), *Shigella* spp. (33), *Streptococcus* Lancefield groups A and G (17), and parasites *Cyclospora*, *Giardia*, and *Cryptosporidium* (23). *Streptococcal*, *staphylococcal*, and typhoid outbreaks seem to be diminishing over time; hepatitis A virus remains static, whereas norovirus and maybe nontyphoidal *Salmonella* are increasing. Multiple foods and multi-ingredient foods were identified most frequently with outbreaks, perhaps because of more frequent hand contact during preparation and serving.

83. Grimes, T. M. (1979). "Observations on *Salmonella* infections of birds."
Aust Vet J **55**(1): 16-18.
http://www.ncbi.nlm.nih.gov/pubmed/435204
During the period 1961 to 1976, 29 species of *Salmonella* other than *Salmonella* Pullorum were isolated from 180 accessions of birds examined at the Animal Research Institute, Yeerongpilly. These birds were submitted to the laboratory from flocks with disease or production problems. *S.* Typhimurium was the most frequently isolated serotype being obtained from 63% of accessions. Outbreaks of systemic salmonellosis occurred most frequently in young birds and although pathological changes were most commonly observed in visceral organs they were also seen in eyes, joints and the brain. Diseases other than salmonellosis were identified in many accessions of birds with systemic or enteric *Salmonella* infections.

84. Groves, P. J., S. M. Sharpe, et al. (2015). "Response of layer and broiler strain chickens to parenteral administration of a live *Salmonella* Typhimurium vaccine."
Poult Sci **94**(7): 1512-1520.
http://www.ncbi.nlm.nih.gov/pubmed/26009756
Responses to the parenteral administration of a live aroA deletion *Salmonella* serovar Typhimurium vaccine given to three brown egg layer strains and two broiler strains were studied. Twenty-five birds of each strain were reared together in floor pens to 6 weeks of age and then moved as individual strains to new floor pens and injected with 10(8) colony forming units (CFU) per bird of the vaccine bacteria intramuscularly or subcutaneously, 10(6) CFU per bird subcutaneously, or phosphate buffered saline (PBS) subcutaneously as a vaccination control. Three birds of one layer strain were injected intramuscularly with 0.5mg/ bird *S.* Typhimurium lipopolysaccharide (LPS) to evaluate whether response was similar for vaccine and endotoxin. Birds were weighed, and rectal temperatures recorded at the time of injection, then observed over 24 hours. Rectal temperatures were measured and blood samples collected for serum IL-6 assay at 3 hours post injection (PI). At 12 hours PI blood samples were drawn for analyses for plasma phosphorus (P), glucose (Glu), cholesterol (Cho), aspartate transaminase (AST), total protein (Ptn) and creatinine kinase (CK). Blood was sampled 14 days PI and tested for serum antibody to *S.* Typhimurium. Vaccination resulted in significant seroconversion by 14 days PI in all strains compared to the controls. The three layer strains exhibited a clinical malaise, evident within 90 minutes of injection, lasting for 12

hours, with complete recovery by 24 hours PI. Only the 10(8) CFU dose given subcutaneously produced an increase in rectal temperature 3 hours PI. Vaccination had no effect on IL-6 or Ptn. All vaccine doses increased P and the higher dose by either route decreased Cho in all bird strains. The 10(8) vaccine dose increased Glu and intramuscular injection markedly elevated CK only in the layer strains. The response was not completely congruous with that to LPS alone. The results highlight the need for consideration of differences in response of bird strain when consideration is given to the parenteral administration of live *Salmonella* vaccines.

85. Gruzdev, N., R. Pinto, et al. (2011). "Effect of desiccation on tolerance of *Salmonella enterica* to multiple stresses."
Appl Environ Microbiol **77**(5): 1667-1673.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3067256/pdf/2156-10.pdf
Reducing the available water in food is a long-established method for controlling bacterial growth in the food industry. Nevertheless, food-borne outbreaks of salmonellosis due to consumption of dry foods have been continuously reported. Previous studies showed that dried *Salmonella* cells acquire high tolerance to heat and ethanol. In order to examine if dehydration also induces tolerance to other stressors, dried *Salmonella enterica* serotype Typhimurium cells were exposed to multiple stresses, and their viability was assessed. Indeed, desiccated *S.* Typhimurium acquired higher tolerance to multiple stressors than nondesiccated cells. The dried cells were significantly more resistant to most stressors, including ethanol (10 to 30%, 5 min), sodium hypochlorite (10 to 100 ppm, 10 min), didecyl dimethyl ammonium chloride (0.05 to 0.25%, 5 min), hydrogen peroxide (0.5 to 2.0%, 30 min), NaCl (0.1 to 1 M, 2 h), bile salts (1 to 10%, 2 h), dry heat (100°C, 1 h), and UV irradiation (125 μW/cm(2), 25 min). In contrast, exposure of *Salmonella* to acetic and citric acids reduced the survival of the dried cells (1.5 log) compared to that of nondesiccated cells (0.5 log). Three other *S. enterica* serotypes, *S.* Enteritidis, *S.* Newport, and *S.* Infantis, had similar stress responses as *S.* Typhimurium, while *S.* Hadar was much more susceptible and gained tolerance to only a few stressors. Our findings indicate that dehydration induces cross-tolerance to multiple stresses in *S. enterica*, demonstrating the limitations of current chemical and physical treatments utilized by the food industry to inactivate food-borne pathogens.

86. Hall, G., M. D. Kirk, et al. (2005). "Estimating foodborne gastroenteritis, Australia."
Emerg Infect Dis **11**(8): 1257-1264.
http://www.ncbi.nlm.nih.gov/pubmed/16102316
We estimated for Australia the number of cases, hospitalizations, and deaths due to foodborne gastroenteritis in a typical year, circa 2000. The total amount of infectious gastroenteritis was measured by using a national telephone survey. The foodborne proportion was estimated from Australian data on each of 16 pathogens. To account for uncertainty, we used simulation techniques to calculate 95% credibility intervals (CrI). The estimate of incidence of gastroenteritis in Australia is 17.2 million (95% confidence interval 14.5-19.9 million) cases per year. We estimate that 32% (95% CrI 24%-40%) are foodborne, which equals 0.3 (95% CrI 0.2-0.4) episodes per person, or 5.4 million (95% CrI 4.0-6.9 million) cases annually in Australia. Norovirus, enteropathogenic *Escherichia coli*, *Campylobacter* spp., and *Salmonella* spp. cause the most illnesses. In addition, foodborne gastroenteritis causes approximately 15,000 (95% CrI 11,000-18,000) hospitalizations and 80 (95% CrI 40-120) deaths annually. This study highlights global public health concerns about foodborne diseases and the need for standardized methods, including assessment of uncertainty, for international comparison.

87. Hall, R. (2002). "Outbreak of gastroenteritis due to *Salmonella* Typhimurium phage type I 35a following consumption of raw egg."
Commun Dis Intell Q Rep **26**(2): 285-287.
http://www.immunise.health.gov.au/internet/main/publishing.nsf/Content/cda-pubs-cdi-2002-cdi2602-cdi2602r.htm

88. Hassan, J. O. and R. Curtiss, 3rd (1997). "Efficacy of a live avirulent *Salmonella* Typhimurium vaccine in preventing colonization and invasion of laying hens by *Salmonella* Typhimurium and *Salmonella* Enteritidis."
Avian Dis **41**(4): 783-791.

http://www.ncbi.nlm.nih.gov/pubmed/9454910
An avirulent live delta cya delta crp *Salmonella* Typhimurium strain chi 3985 that precludes colonization and invasion of chickens by homologous and heterologous *Salmonella* serotypes was evaluated for its long-term protection efficacy. Chickens vaccinated orally at 2 and 4 wk of age were assessed for protection against oral challenge with wild-type *S.* Typhimurium and *Salmonella* Enteritidis strains at 3, 6, 9, and 12 mo of age. A comparison of *Salmonella* isolation from vaccinated and nonvaccinated layers after challenge with *S.* Typhimurium or *S.* Enteritidis showed that delta cya delta crp *S*. Typhimurium chi 3985 induced excellent protection against intestinal, visceral, reproductive tract, and egg colonization, invasion, and/or contamination by *Salmonella*. The duration of protection lasted for 11 mo after vaccination, at which time the experiment was terminated. *S*. Enteritidis and *S*. Typhimurium were isolated from the yolk, albumen, and shells of eggs laid by nonvaccinated chickens challenged with *Salmonella*. *S.* Typhimurium caused pathological lesions in nonvaccinated chickens, whereas vaccinated and nonvaccinated chickens challenged with *S*. Enteritidis showed no pathological lesion in the visceral and reproductive organs. Vaccination with chi 3985 prevented transmission of *S*. Typhimurium or *S*. Enteritidis into eggs laid by vaccinated layers with no effect on egg production. To our knowledge, this is the first publication confirming that vaccination with live avirulent *Salmonella* can induce long-term protection against *Salmonella* infection in layers.

89. Hennebry, S. C., L. C. Sait, et al. (2012). "*Salmonella* Typhimurium's transthyretin-like protein is a host-specific factor important in fecal survival in chickens."
PLoS One **7**(12): e46675.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3528726/pdf/pone.0046675.pdf
The transthyretin-like protein (TLP) from *Salmonella enterica* subspecies I is a periplasmic protein with high level structural similarity to a protein found in mammals and fish. In humans, the protein homologue, transthyretin, binds and carries retinol and thyroxine, and a series of other, unrelated aromatic compounds. Here we show that the amino acid sequence of the TLP from different species, subspecies and serovars of the *Salmonella* genus is highly conserved and demonstrate that the TLP gene is constitutively expressed in *S*. Typhimurium and that copper and other divalent metal ions severely inhibit enzyme activity of the TLP, a cyclic amidohydrolase that hydrolyses 5-hydroxyisourate (5-HIU). In order to determine the in vivo role of the *S*. Typhimurium TLP, we constructed a strain of mouse-virulent *S*. Typhimurium SL1344 bearing a mutation in the TLP gene (SL1344 DeltayedX). We assessed the virulence of this strain via oral inoculation of mice and chickens. Whilst SL1344 DeltayedX induced a systemic infection in both organisms, the bacterial load detected in the faeces of infected chickens was significantly reduced when compared to the load of *S*. Typhimurium SL1344. These data demonstrate that the TLP gene is required for survival of *S*. Typhimurium in a high uric acid environment such as chicken faeces, and that metabolic traits of Salmonellae in natural and contrived hosts may be fundamentally different. Our data also highlight the importance of using appropriate animal models for the study of bacterial pathogenesis especially where host-specific virulence factors or traits are the subject of the study.

90. Hennessy, T. W., C. W. Hedberg, et al. (1996). "A National Outbreak of *Salmonella* Enteritidis Infections from Ice Cream."
New Engl J Med **334**(20): 1281-1286.
http://www.nejm.org/doi/pdf/10.1056/NEJM199605163342001
In September 1994, the Minnesota Department of Health detected an increase in the number of reports of *Salmonella* Enteritidis infections. After a case–control study implicated a nationally distributed brand of ice cream (Schwan's) in the outbreak, the product was recalled and further epidemiologic and microbiologic investigations were conducted. We defined an outbreak-associated case of *S.* Enteritidis infection as one in which *S*. Enteritidis was cultured from a person who became ill in September or October 1994. We established national surveillance and surveyed customers of the implicated manufacturer. The steps involved in the manufacture of ice cream associated with cases of *S*. Enteritidis infection were compared with those of products not known to be associated with infection matched for the date of manufacture. Cultures for bacteria were obtained from ice cream samples, the ice cream plant, and tanker trailers that had transported the ice cream base (premix) to the plant. We estimate that *S.* Enteritidis gastroenteritis developed in 224,000 persons in the United

States after they ate Schwan's ice cream. The attack rate for consumers was 6.6 percent. Ice cream associated with infection contained a higher percentage of premix that had been transported by tanker trailers that had carried nonpasteurized eggs immediately before (P=0.02). *S*. Enteritidis was isolated from 8 of 266 ice cream products (3 percent), but not from environmental samples obtained from the ice cream plant (n =157) or tanker trailers (n =204). This nationwide outbreak of salmonellosis was most likely the result of contamination of pasteurized ice cream premix during transport in tanker trailers that had previously carried nonpasteurized liquid eggs containing *S*. Enteritidis. To prevent further outbreaks, food products not destined for repasteurization should be transported in dedicated containers.

91. Hogue, A. T., E. D. Ebel, et al. (1997). "Surveys of *Salmonella* Enteritidis in unpasteurized liquid egg and spent hens at slaughter."
J Food Prot **60**(10): 1194-1200.
http://www.ingentaconnect.com/content/iafp/jfp/1997/00000060/00000010/art00007
In a 1995 national survey of 937 unpasteurized liquid egg samples collected in breaker plants, 179 of 937 samples (19%) were Salmonella enterica serotype Enteritidis (*S*. Enteritidis) positive. The proportion of unpasteurized liquid egg samples positive for *S*. Enteritidis was highest in the Northern Region where 106 of 267 samples collected (40%) were *S*. Enteritidis-positive. These Northern Region results were over three times the *S*. Enteritidis prevalence detected from the other three regions, whose results ranged between 10% and 12% *S*. Enteritidis-positive samples. In a 1995 national survey of spent hens at slaughter, 136 of 305 flocks (45%) had at least one *S*. Enteritidis-positive pooled sample detected. Flock prevalence was highest in the Northern and Central Regions (64% and 40%, respectively): Southeastern and Western Regional flock prevalence levels were much lower (17% and 23%, respectively). A comparison of the 1991 and 1995 unpasteurized liquid egg and spent hen results suggest there has been no decline in *S*. Enteritidis occurrence in the commercial egg industry between 1991 and 1995. *Salmonella* Enteritidis phage type 4 was detected in the 1995 surveys of both spent hens and unpasteurized liquid egg but was not found in either survey in 1991. With the exception of one liquid egg sample from the Southeastern Region, *S*. Enteritidis phage type 4 was found only in the Western Region of the U.S. *S*. Enteritidis phage type 4 has emerged in the egg industry in the Western U.S. concurrently with an increase in the number of sporadic human phage type 4 isolates in California and Utah.

92. Holt, P. S. (1993). "Effect of induced molting on the susceptibility of White Leghorn hens to a *Salmonella* Enteritidis infection."
Avian Dis **37**(2): 412-417.
http://www.ncbi.nlm.nih.gov/pubmed/8363506
Older white leghorn hens (more than 52 weeks old) were induced to molt using a 14-day feed-removal protocol. On day 4 of feed removal, groups of hens were infected with varying 10-fold dilutions of *Salmonella* Enteritidis, and these hens were examined for *S.* Enteritidis intestinal shedding 7 days later. Molting hens infected with a 10(-2) dilution of *S.* Enteritidis shed 3-4 logs more of the organism at 7 days postinfection than the unmolted group receiving a similar dose. The mean infectious dose (ID50) for S. enteritidis in unmolted hens ranged from 0.65 x 10(4) to 5.6 x 10(4), whereas in molting hens the ID50 was found to be less than 10(1), a 2-3 log increase in the susceptibility of the hens to the organism.

93. Holt, P. S., R. H. Davies, et al. (2011). "The impact of different housing systems on egg safety and quality."
Poult Sci **90**(1): 251-262.
http://ps.oxfordjournals.org/content/90/1/251.full.pdf
A move from conventional cages to either an enriched cage or a noncage system may affect the safety or quality, or both, of the eggs laid by hens raised in this new environment. The safety of the eggs may be altered either microbiologically through contamination of internal contents with *Salmonella enterica* serovar Enteritidis (*Salmonella* Enteritidis) or other pathogens, or both, or chemically due to contamination of internal contents with dioxins, pesticides, or heavy metals. Quality may be affected through changes in the integrity of the shell, yolk, or albumen along with changes in function, composition, or nutrition. Season, hen breed, flock age, and flock disease-vaccination status also interact to affect egg safety and

quality and must be taken into account. An understanding of these different effects is prudent before any large-scale move to an alternative housing system is undertaken.

94. Holt, P. S., C. J. Geden, et al. (2007). "Isolation of *Salmonella enterica* serovar Enteritidis from houseflies (Musca domestica) found in rooms containing *Salmonella* serovar Enteritidis-challenged hens."
Appl Environ Microbiol **73**(19): 6030-6035.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2075029/pdf/0803-07.pdf
Houseflies (Musca domestica) released into rooms containing hens challenged with *Salmonella enterica* serovar Enteritidis (*Salmonella* serovar Enteritidis) rapidly became contaminated with *Salmonella* serovar Enteritidis. Forty to 50% of the flies were contaminated at 48 h, and the percentage increased to 50 to 70% at 4 and 7 days postexposure and then decreased to 30% at day 15. Initial attempts at recovering surface organisms for culture using an aqueous rinse were largely unsuccessful, while cultures of internal contents readily recovered *Salmonella* serovar Enteritidis. However, when 0.5% detergent was incorporated into the rinse, high recovery levels of bacteria were observed from both external and internal culture regimens, indicating equal distribution of the organism on and in the fly and a tighter interaction of the organism with the host than previously thought. *Salmonella* serovar Enteritidis was isolated routinely from the fly gut, on rare occasions from the crop, and never from the salivary gland. Feeding contaminated flies to hens resulted in gut colonization of a third of the birds, but release of contaminated flies in a room containing previously unchallenged hens failed to result in colonization of any of the subject birds. These results indicate that flies exposed to an environment containing *Salmonella* serovar Enteritidis can become colonized with the organism and might serve as a source for transmission of *Salmonella* serovar Enteritidis within a flock situation.

95. Holt, P. S. and R. E. Porter, Jr. (1993). "Effect of induced molting on the recurrence of a previous *Salmonella* Enteritidis infection."
Poult Sci **72**(11): 2069-2078.
http://www.ncbi.nlm.nih.gov/pubmed/8265497
Previous work in the authors' laboratory had shown that hens infected with *Salmonella* Enteritidis (SE) during the feed removal phase of an induced molt shed significantly more SE and more readily transmitted SE to uninfected hens in adjacent cages when compared with unmolted hens. A study was conducted to examine the effect of induced molting on the recurrence and horizontal transmission of a previous SE infection. Hens aged 59 and 69 wk in Trials 1 and 2, respectively, were infected with SE and then molted 21 days later. In Trial 1, more molted hens were SE-culture-positive on Days 38 (P < or = .005) and 45 (P < or = .005) postinfection, and these hens shed more SE on these days (P < or = .05 and P < or = .005, respectively) than unmolted hens. Horizontal transmission of SE to previously uninfected but contact-exposed hens in adjacent cages was also higher in the molted group than the unmolted group on Days 38 (P < or = .05) and 45 (P < or = .001). Molted, contact-exposed hens also shed significantly more SE than unmolted hens. In Trial 2, the molted infected hens shed progressively more SE than the unmolted hens but the differences were not significant. However, more molted contact-exposed hens became SE-positive at Day 31 (P < or = .05) and 38 (P < or = .005) and also shed more SE on these days (P < or = .05 and P < or = .01), respectively) than the unmolted hens. Serum and intestinal antibody titers to SE were also examined in Trial 2. Molting appeared to exert no effect on the serum SE titers, but antibody titers in the alimentary tract were lower in the molted hens than the unmolted hens on Days 45 (P < or = .005) and 52 (P < or = .05). In Trial 1, three of eight molted directly infected hens and two of eight molted contact-exposed hens produced any SE-contaminated eggs. In Trial 2, no SE-contaminated eggs were produced.

96. Hope, B. K., R. Baker, et al. (2002). "An overview of the *Salmonella* Enteritidis risk assessment for shell eggs and egg products."
Risk Anal **22**(2): 203-218.
http://www.ncbi.nlm.nih.gov/pubmed/12022671
This article summarizes a quantitative microbial risk assessment designed to characterize the public health impact of consumption of shell eggs and egg products contaminated with *Salmonella* Enteritidis (SE). This risk assessment's objectives were to:
(1) establish the baseline risk of foodborne illness from SE,

(2) identify and evaluate potential risk mitigation strategies, and
(3) identify data gaps related to future research efforts. The risk assessment model has five modules. The Egg Production module estimates the number of eggs produced that are SE-contaminated. Shell Egg Processing, Egg Products Processing, and Preparation & Consumption modules estimate the increase or decrease in the numbers of SE organisms in eggs or egg products as they pass through storage, transportation, processing, and preparation. A Public Health Outcomes module then calculates the incidence of illnesses and four clinical outcomes, as well as the cases of reactive arthritis associated with SE infection following consumption. The baseline model estimates an average production of 2.3 million SE-contaminated shell eggs/year of the estimated 69 billion produced annually and predicts an average of 661,633, human illnesses per year from consumption of these eggs. The model estimates approximately 94% of these cases recover without medical care, 5% visit a physician, an additional 0.5% are hospitalized, and 0.05% result in death. The contribution of SE from commercially pasteurized egg products was estimated to be negligible. Five mitigation scenarios were selected for comparison of their individual and combined effects on the number of human illnesses. Results suggest that mitigation in only one segment of the farm-to-table continuum will be less effective than several applied in different segments. Key data gaps and areas for future research include the epidemiology of SE on farms, the bacteriology of SE in eggs, human behavior in food handling and preparation, and human responses to SE exposure.

97. Horchner, P. M., D. Brett, et al. (2006). "HACCP-based approach to the derivation of an on-farm food safety program for the Australian red meat industry."
Food Control **17**(7): 497-510.
http://www.sciencedirect.com/science/article/pii/S0956713505000617
The standard Codex HACCP approach was modified to allow a hazard analysis to be conducted at an industry level which could then be used to derive appropriate on-farm food safety control measures for cattle, sheep and goat production in Australia. Scientific information from a through chain risk profile of the red meat industry was used as a major resource for the hazard analysis. The process resulted in the identification of critical control points for control of bovine spongioform encephalopathy (BSE), prevention of violations of maximum residue limits with agricultural and veterinary chemicals and infection with Cysticercus bovis (Beef Measles). By applying this HACCP-based approach it was determined that the application of a simple set of good agricultural practices (GAP) on-farm would be effective in ensuring low risk. It was, therefore, concluded that on-farm food safety schemes may not warrant full HACCP plans at the individual enterprise level as long as appropriate GAP is in place. The results provide red meat producers with the elements of a HACCP-based food safety scheme that is scientifically justifiable, understandable and realistic to apply which are essential elements that underpin successful implementation and compliance by industry. Subsequently, an on-farm food safety program has been developed to provide an appropriate level of protection for consumers as well as to protect Australia's trade from food safety-related issues.

98. Horchner, P. M. and A. M. Pointon (2011). "HACCP-based program for on-farm food safety for pig production in Australia."
Food Control **22**(10): 1674-1688.
http://www.sciencedirect.com/science/article/pii/S0956713511001198
The standard Codex HACCP approach was modified to allow a hazard analysis and critical control point determination to be conducted at an industry level and then used to determine the appropriate on-farm food safety control measures for pig production in Australia. A detailed risk-based profile with hazard identification, hazard characterisation and levels of microbial contamination for production and primary processing was used as a major technical resource to inform HACCP determinations. The process resulted in the identification of Critical Control Points for control of a specific physical hazard (non-recovered broken needles) and prevention of violations of Maximum Residue Limits with agricultural and veterinary chemicals. In relation to the identified microbiological hazards, it was noted that there are numerous Good Agricultural Practices (GAP) to prevent and/or reduce their risk and these controls would also need to be a feature of any on-farm food safety program. By applying a HACCP-based approach it was determined that the application of a set of Good Agricultural Practices on-farm would be effective in ensuring low risk. It was, therefore,

concluded that on-farm food safety programs may not warrant full (i.e. Codex compliant) HACCP plans at the individual enterprise level provided appropriate GAP is in place. The results provide pig producers and the Australian pig industry with the elements of a HACCP-based food safety system that are scientifically justifiable, understandable and realistic to apply. These features are essential elements that underpin successful implementation and compliance by industry.

99. Humphrey, T. J. (1990). "Growth of salmonellas in intact shell eggs: influence of storage temperature."
Vet Rec **126**(12): 292.
http://www.ncbi.nlm.nih.gov/pubmed/2188413

100. Humphrey, T. J., H. Chart, et al. (1991). "The influence of age on the response of SPF hens to infection with *Salmonella* Enteritidis PT4."
Epidemiol Infect **106**(1): 33-43.
http://www.ncbi.nlm.nih.gov/pubmed/1825197
When Specific Pathogen-Free hens were infected with *Salmonella* Enteritidis PT4 by direct administration into the crop, the age of the bird at infection was found to have an effect on both pathogenesis and antibody response. Birds at 20 weeks of age showed no adverse signs and developed high titres of antibodies of the IgM class, while those which were 1 year old at infection developed relatively little antibody and had acute septicaemia, with 6 of 10 birds either dying or having to be humanely destroyed. The implication of these results for the control of *Salmonella* infections in poultry is discussed.

101. Hundy, R. L. and S. Cameron (2002). "An outbreak of infections with a new *Salmonella* phage type linked to a symptomatic food handler."
Commun Dis Intell Q Rep **26**(4): 562-567.
http://www.ncbi.nlm.nih.gov/pubmed/12549524
In December 2001, the South Australian Communicable Disease Control Branch investigated an outbreak of gastrointestinal illness linked to a Korean style restaurant in metropolitan Adelaide. Twenty-eight people were identified as having experienced gastrointestinal symptoms subsequent to dining at the restaurant between 9 and 12 December 2001. A case-control study implicated mango pudding dessert (OR 16.67 95% CI 2.03-177.04) and plain chicken (OR 10.67 95% CI 1.04-264.32). Nineteen cases and one food handler submitted faecal specimens that grew *Salmonella* Typhimurium 64var. Two samples of mango pudding and one sample of pickled Chinese cabbage also grew *Salmonella* Typhimurium 64var. The infected food handler reported an onset of illness 2 days before cases first reported eating at the restaurant. The food handler's only role was to prepare the mango pudding dessert in an area external to the restaurant's kitchen. Illness was strongly associated with consumption of a contaminated mango pudding dessert, with contamination most likely resulting from the symptomatic and culture positive food handler who prepared the dish. This outbreak demonstrates the importance of excluding symptomatic food handlers, and the need for appropriately informing and educating food handlers regarding safe food handling procedures. Restaurants with staff and management from non-English speaking backgrounds should be specifically targeted for education that is both culturally sensitive and language specific.

102. Jardine, A., K. A. Ressler, et al. (2011). "An outbreak of *Salmonella* Typhimurium 108/170 at a privately catered barbeque at a Sydney sports club."
Foodborne Pathog Dis **8**(11): 1215-1219.
http://www.ncbi.nlm.nih.gov/pubmed/21790276
An outbreak of gastrointestinal illness was identified among attendees at a large community barbeque at a Sydney sports club on 30 January 2009. A retrospective cohort study was initiated, and attendees were identified through hospital emergency department gastroenteritis presentations, snowball recruitment through known cases, responders to linguistically specific press, and those returning to the venue the next week. A symptom and food history was collected from attendees, and stool samples were provided for microbiological investigation. An environmental investigation and trace back of implicated foods was also undertaken. Attendance estimates at the barbeque ranged from 100 to 180, and the food was prepared by a family that was not registered as a food business. Seventy

one of the 87 attendees identified met the case definition. Thirty attendees (42%) had laboratory confirmed *Salmonella* Typhimurium phage-type 108/170, all with the same multilocus variable number of tandem repeat analysis typing. Burden of illness was high with 76% of cases seeking medical attention and 18% admitted to hospital. Microbiological evidence confirmed that a number of food items were contaminated with *Salmonella* Typhimurium 108/170, with the raw egg mayonnaise used in a Russian salad being the most likely primary food vehicle (adjusted odds ratio=10.3 [95% confidence interval 1.79-59.5]). Further, having Russian salad on the plate even if it was not consumed increased the relative risk of illness, thus suggesting that other food items may have been contaminated when they came into contact with it on the plate. This *Salmonella* outbreak highlighted the risks associated with the improper handling of food in private residences, which are then sold at a large public event.

103. Jay-Russell, M. T., A. F. Hake, et al. (2014). "Prevalence and characterization of *Escherichia coli* and *Salmonella* strains isolated from stray dog and coyote feces in a major leafy greens production region at the United States-Mexico border."
PLoS One **9**(11): e113433.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4239069/pdf/pone.0113433.pdf
In 2010, Romaine lettuce grown in southern Arizona was implicated in a multi-state outbreak of *Escherichia coli* O145:H28 infections. This was the first known Shiga toxin-producing *E. coli* (STEC) outbreak traced to the southwest desert leafy green vegetable production region along the United States-Mexico border. Limited information exists on sources of STEC and other enteric zoonotic pathogens in domestic and wild animals in this region. According to local vegetable growers, unleashed or stray domestic dogs and free-roaming coyotes are a significant problem due to intrusions into their crop fields. During the 2010-2011 leafy greens growing season, we conducted a prevalence survey of STEC and *Salmonella* presence in stray dog and coyote feces. Fresh fecal samples from impounded dogs and coyotes from lands near produce fields were collected and cultured using extended enrichment and serogroup-specific immunomagnetic separation (IMS) followed by serotyping, pulsed-field gel electrophoresis (PFGE), and antimicrobial susceptibility testing. A total of 461 fecal samples were analyzed including 358 domestic dog and 103 coyote fecals. STEC was not detected, but atypical enteropathogenic *E. coli* (aEPEC) strains comprising 14 different serotypes were isolated from 13 (3.6%) dog and 5 (4.9%) coyote samples. *Salmonella* was cultured from 33 (9.2%) dog and 33 (32%) coyote samples comprising 29 serovars with 58% from dogs belonging to Senftenberg or Typhimurium. PFGE analysis revealed 17 aEPEC and 27 *Salmonella* distinct pulsotypes. Four (22.2%) of 18 aEPEC and 4 (6.1%) of 66 Salmonella isolates were resistant to two or more antibiotic classes. Our findings suggest that stray dogs and coyotes in the desert southwest may not be significant sources of STEC, but are potential reservoirs of other pathogenic *E. coli* and *Salmonella*. These results underscore the importance of good agriculture practices relating to mitigation of microbial risks from animal fecal deposits in the produce production area.

104. Jay, S., D. Davos, et al. (2012). *Salmonella*
Foodborne Microorganisms of public health significance.
A. D. Hocking. Riverwood, NSW, Australia, Ligare Book Printers**:** 207-268.

105. Jiang, C., K. S. Shaw, et al. (2015). "Climate change, extreme events and increased risk of salmonellosis in Maryland, USA: Evidence for coastal vulnerability."
Environ Int **83**: 58-62.
http://www.sciencedirect.com/science/article/pii/S0160412015001361#
BACKGROUND: *Salmonella* is a leading cause of acute gastroenteritis worldwide. Patterns of salmonellosis have been linked to weather events. However, there is a dearth of data regarding the association between extreme events and risk of salmonellosis, and how this risk may disproportionately impact coastal communities.
METHODS: We obtained *Salmonella* case data from the Maryland Foodborne Diseases Active Surveillance Network (2002-2012), and weather data from the National Climatic Data Center (1960-2012). We developed exposure metrics related to extreme temperature and precipitation events using a 30year baseline (1960-1989) and linked them with county-level salmonellosis data. Data were analyzed using negative binomial Generalized Estimating Equations.

RESULTS: We observed a 4.1% increase in salmonellosis risk associated with a 1unit increase in extreme temperature events (incidence rate ratio (IRR):1.041; 95% confidence interval (CI):1.013-1.069). This increase in risk was more pronounced in coastal versus non-coastal areas (5.1% vs 1.5%). Likewise, we observed a 5.6% increase in salmonellosis risk (IRR:1.056; CI:1.035-1.078) associated with a 1unit increase in extreme precipitation events, with the impact disproportionately felt in coastal areas (7.1% vs 3.6%).
CONCLUSIONS: To our knowledge, this is the first empirical evidence showing that extreme temperature/precipitation events-that are expected to be more frequent and intense in coming decades-are disproportionately impacting coastal communities with regard to salmonellosis. Adaptation strategies need to account for this differential burden, particularly in light of ever increasing coastal populations.

106. Jimenez, M., J. H. Siller, et al. (2007). "Bidirectional *Salmonella enterica* serovar Typhimurium transfer between bare/glove hands and green bell pepper and its interruption."
Int J Environ Health Res **17**(5): 381-388.
http://www.ncbi.nlm.nih.gov/pubmed/17924266
The aim of this study was to quantify the amount of *Salmonella enterica* serovar Typhimurium transferred from volunteers' hands (bare or gloved) to green bell peppers and vice versa; and to assess the effectiveness of hand hygiene techniques. The highest and lowest percentages of bacterial transfer were achieve from green bell peppers to gloved hands (46.56%) and from bare hands to green bell peppers (0.21%), respectively. The highest and lowest Log10 reductions of *S.* Typhimurium were achieved by the combination of hand washing and alcohol-based gel (4.38 Log10) and iodine solution (2.08 Log10), respectively. This study provides important information concerning the transfer's efficiency of *S*. Typhimurium from hands to fresh produce and from fresh produce to hands. The study also showed that gloved hands, could be a mean of transfer of *S.* Typhimurium between green peppers and hands, and the best hand hygiene technique was the combination of hand washing and alcohol-based gel.

107. Jin, T. Z., J. B. Gurtler, et al. (2013). "Development of antimicrobial coatings for improving the microbiological safety and quality of shell eggs."
J Food Prot **76**(5): 779-785.
http://www.ncbi.nlm.nih.gov/pubmed/23643119
This study was conducted to develop antimicrobial coatings to decontaminate and prevent cross-contamination of shell eggs. Egg shells were inoculated with nalidixic acid-resistant *Salmonella enterica* Enteritidis strains OB030832, OB040159, and C405 and treated with antimicrobial coatings. Polylactic acid served as a nonedible polymer, and chitosan served as an edible polymer carrier of natural antimicrobials, including nisin, allyl isothiocyanate (AIT), lauric arginate ester (LAE), and organic acids. Increases of AIT concentrations or addition of nisin to AIT in either the polylactic acid or chitosan coating solutions resulted in greater reductions of *Salmonella*. Chitosan coatings with 0.1, 0.5, and 1.0% LAE reduced *Salmonella* by 1.7, 2.5, and 5.2 log CFU/cm(2), respectively. Shell eggs treated with 1.0 and 0.5% LAE in chitosan coatings had nondetectable *Salmonella* cells (< 0.5 log CFU/cm(2)) after 3 and 7 days of storage at 7 degrees C, respectively, and no outgrowth was observed up to 28 days. Coating treatments significantly reduced weight loss of shell eggs during 12 weeks of storage at 7 or 4 degrees C. This study demonstrates an alternative and effective intervention technology for decontaminating shell eggs and provides an alternative approach to reduce possible recalls and outbreaks associated with pathogen contamination on shell eggs and in egg products.

108. Jones, D. R. (2010). "Microbiological and physical quality changes in vacuum loader cups associated with the use of various sanitizing compounds."
Poult Sci **89**(3): 564-569.
http://ps.oxfordjournals.org/content/89/3/564.long
Studies were conducted to determine the effects of various sanitizing compounds on the microbial and physical quality of shell egg processing vacuum loader cups. The sanitizing compounds used were as follows: sterile, distilled water; 200 microL/L of sodium hypochlorite; 200 microL/L of calcium hypochlorite; and 200 microL/L of peracetic acid. In the microbial inoculation study, cups were inoculated with *Enterobacter cloacae* because it was the most common isolate from a commercial study examining the flora found on vacuum loader cups.

In all 3 replicates, aerobic plate counts and *Enterobacteriaceae* levels were similar for the clean control cups and the cups from the 2 chlorine treatments. Physical quality was measured via serial static compression testing using texture profile analysis. The serial compression mimicked the movement of the vacuum loader cups on the processing line. The strength of the vacuum loader cups was enhanced with exposure to any sanitizer treatment, including distilled water, compared with the controls throughout the 20 applications of the sanitizers. Durometer measurements were not consistent in monitoring vacuum loader cup quality and were determined to not be effective assessments for this application. The use of 200 microL/L of sodium hypochlorite or 200 microL/L of calcium hypochlorite successfully reduced microbial contaminants, had a positive effect on vacuum loader cup physical quality, and should be considered when developing sanitation programs for shell egg processing facilities.

109. Jones, D. R. and K. E. Anderson (2013). "Housing system and laying hen strain impacts on egg microbiology."
Poult Sci **92**(8): 2221-2225.
http://ps.oxfordjournals.org/content/92/8/2221.long
Alternative hen housing is becoming more commonplace in the egg market. However, a complete understanding of the implications for alternative housing systems on egg safety has not been achieved. The current study examines the impact of housing Hy-Line Brown, Hy-Line Silver Brown, and Barred Plymouth Rock hens in conventional cage, cage-free, and free range egg production systems on shell microbiology. Eggs were collected at 4 sampling periods. Egg shell emulsion pools were formed and enumerated for total aerobic organisms, Enterobacteriaceae, and yeast and mold counts. Hy-Line Brown and Hy-Line Silver Brown hens produced eggs with significantly ($P < 0.05$ and 0.001, respectively) different levels of aerobic organisms dependent on housing system. Eggs from conventional cages had significantly different ($P < 0.05$) levels of aerobic contamination in relation to hen strain with Hy-Line Silver Brown having the greatest (4.57 log cfu/mL). Hy-Line Brown and Barred Plymouth Rock hens produced eggs with significantly different ($P < 0.01$) levels of *Enterobacteriaceae* among housing systems with conventional caged eggs having the lowest level of contamination for the hen strains. There were no differences within each strain among housing systems for yeast and mold contamination. The study shows that hen strain has an effect on egg microbial levels for various housing systems, and egg safety should be considered when making hen strain selections for each housing system.

110. Jones, D. R., K. E. Anderson, et al. (2002). "Microbial contamination in inoculated shell eggs: I. Effects of layer strain and hen age."
Poult Sci **81**(5): 715-720.
http://ps.oxfordjournals.org/content/81/5/715.long
Three Ottawa control strains and a current commercial laying stock were reared and housed under identical environmental and management conditions. Eggs were collected from each strain when hens were 32, 45, 58, 71, and 84 wk of age. The eggs were inoculated with *Salmonella* Enteritidis (SE), *Pseudomonas fluorescens* (PF), or a combination of the two. After storage at 26 C, bacterial counts were obtained from the exterior shell surfaces (rinse), air cell, egg contents, and shell structure. SE and PF survived at different rates on the shell surface with as much as a 1 log difference during a given collection period. Egg content counts tended to be higher than eggshell counts in PF, whereas the opposite was true for SE. These data suggest that PF is a primary invader of eggs that is more capable of contaminating egg contents through the shell membranes than SE. The PF and SE data suggest that bacterial contamination of air cells, shells, and egg contents is more easily achieved in eggs from older hens than from younger hens. There were also differences between the strains. Control Strain 10 consistently maintained a lower level of contamination for both organisms in each sampling location. The overall results of this study suggest that genetic selection has altered the ability of eggs to resist microbial contamination and that screening for microbial integrity should be considered in the selection process among the laying egg breeders.

111. Jones, D. R., K. E. Anderson, et al. (2012). "Prevalence of coliforms, *Salmonella*, *Listeria*, and *Campylobacter* associated with eggs and the environment of conventional cage and free-range egg production."

Poult Sci **91**(5): 1195-1202.
http://ps.oxfordjournals.org/content/91/5/1195.long
There is a desire by US consumers for eggs produced by hens in alternative production systems. As the retail shell-egg market offers these products to accommodate consumer demands, additional information is needed to ensure processing methodologies result in safe eggs from all egg sources. A study was conducted to determine if there were differences in the prevalence of coliforms, *Salmonella*, *Listeria*, and *Campylobacter* on and within eggs and in the environment of a sister flock of conventional cage and free-range laying hens. Microbial sampling occurred approximately every 6 wk between 20 and 79 wk of age. A random sampling of typical coliform colonies produced 371 viable isolates for biochemical identification. Twenty-nine genera or species of bacteria were identified. There was a significantly greater ($P < 0.0001$) prevalence of *Campylobacter* in the free-range nest box swabs compared with that in the free-range grass and conventional cage swab samples (number of positives: 8 nest box, 1 grass, 0 cage). Seven isolates of *Listeria innocua* were detected with no significant difference in prevalence between the treatments. Isolates were associated with eggshells (2 free-range floor, 1 cage) and the free-range environment (2 nest box, 2 grass). There were 21 *Salmonella* isolates detected between all sample locations, with no significant difference in the prevalence of *Salmonella* detection between the treatments. Additional studies are needed to fully understand the effect of alternative production methods on the prevalence of pathogens and coliforms associated with nest-run eggs and the production environment.

112. Jones, D. R., N. A. Cox, et al. (2015). "Microbiological impact of three commercial laying hen housing systems."
Poult Sci **94**(3): 544-551
http://ps.oxfordjournals.org/content/94/3/544.full.pdf
Hen housing for commercial egg production continues to be a societal and regulatory concern. Controlled studies have examined various aspects of egg safety, but a comprehensive assessment of commercial hen housing systems in the US has not been conducted. The current study is part of a holistic, multidisciplinary comparison of the diverse aspects of commercial conventional cage, enriched colony cage, and cage-free aviary housing systems and focuses on environmental and egg microbiology. Environmental swabs and eggshell pools were collected from all housing systems during 4 production periods. Total aerobes and coliforms were enumerated, and the prevalence of *Salmonella* and *Campylobacter* spp. was determined. Environmental aerobic and coliform counts were highest for aviary drag swabs (7.5 and 4.0 log cfu/mL, respectively) and enriched colony cage scratch pad swabs (6.8 and 3.8 log cfu/mL, respectively). Aviary floor and system wire shell pools had the greatest levels of aerobic contamination for all eggshell pools (4.9 and 4.1 log cfu/mL, respectively). Hens from all housing systems were shedding *Salmonella* spp. (89-100% of manure belt scraper blade swabs). The dry belt litter removal processes for all housing systems appear to affect *Campylobacter* spp. detection (0-41% of manure belt scraper blade swabs) considering detection of *Campylobacter* spp. was much higher for other environmental samples. Aviary forage area drag swabs were 100% contaminated with *Campylobacter* spp., whereas enriched colony cage scratch pads had a 93% positive rate. There were no differences in pathogen detection in the shell pools from the 3 housing systems. Results indicate egg safety is enhanced when hens in alternative housing systems use nest boxes. Additionally, current outcomes indicate the use of scratch pads in hen housing systems needs to be more thoroughly investigated for effects on hen health and egg safety.

113. Jones, D. R., K. C. Lawrence, et al. (2010). "Modified pressure imaging for egg crack detection and resulting egg quality."
Poult Sci **89**(4): 761-765.
http://ps.oxfordjournals.org/content/89/4/761.long
Cracks in the shell surface compromise the primary barrier for external microbial contamination of the egg. Microcracks are very small cracks in the shell surface that are difficult to detect by human graders. New technology has been developed that uses modified pressure and imaging to detect microcracks in eggs. Research has shown the system to have an accuracy of 99.6% in detecting both cracked and intact eggs. A study was undertaken to determine if quality differences existed between modified pressure imaged and control eggs

during extended cold storage. Three replicates were conducted with eggs stored at 4 degrees C for 5 wk with weekly quality testing. The physical quality factors monitored were Haugh units, albumen height, egg weight, shell strength, vitelline membrane strength and elasticity, and whole egg total solids. All measurements were conducted on individual eggs (12/treatments per replicate) each week with the exception of whole egg solids, which were determined from 3 pools (4 eggs each)/treatment per replicate each week. Percentage of whole egg total solids was the only significant difference ($P < 0.05$) between treatments (23.65% modified pressure imaged and 23.47% control). There was a significant difference ($P < 0.05$) for egg weight between replicates (60.82, 58.02, and 60.58 g for replicates 1, 2, and 3, respectively). Therefore, imaging eggs in the modified pressure system for microcrack detection did not alter egg quality during extended cold storage. Utilizing the modified pressure crack detection technology would result in fewer cracked eggs reaching the consumer, consequently enhancing food safety without affecting product quality.

114. Jones, D. R. and M. T. Musgrove (2008). "Identification of enterobacteriaceae on vacuum loaders in shell egg processing."
Poult Sci **87**(8): 1678-1681.
http://ps.oxfordjournals.org/content/87/8/1678.long
Cleaning and sanitation are paramount in food processing. Gaining an understanding of the microbial populations present in a processing facility can help in the development of effective and efficient cleaning. The current study was undertaken to gain a better understanding of the *Enterobacteriaceae* present on vacuum loader cups used in shell egg processing to transfer nest run eggs to the processing line. Twenty cups were rinsed on each of 3 visits to both an off-line operation and a mixed operation. A total of 442 *Enterobacteriaceae* isolates were biochemically identified from vacuum loader cup rinses. The predominant genera isolated from the 2 facilities were *Enterobacter*, *Klebsiella*, *Escherichia*, *Citrobacter*, and *Serratia*. The primary organisms from the off-line facility were *Klebsiella oxytoca*, *Enterobacter amnigenus* 2, and *Klebsiella pneumoniae*. The isolates found in the greatest proportion from the mixed operation were *Enterobacter cloacae* and *Klebsiella oxytoca*. A total of 18 genera were recovered from the 2 facilities, with 9 being present in both processing facilities. The findings of this study can be used in assessing the sources of bacterial contamination in egg processing and in developing more effective, targeted cleaning programs for processing equipment and facilities.

115. Jones, D. R., J. K. Northcutt, et al. (2003). "Survey of shell egg processing plant sanitation programs: effects on egg contact surfaces."
J Food Prot **66**(8): 1486-1489.
http://www.ncbi.nlm.nih.gov/pubmed/12929843
Sanitation standard operating procedures (SSOPs) are an integral component of process control and are often the first step in the implementation of food safety regulations. The objective of this study was to assess and compare the efficacies of sanitation programs used in a variety of shell egg processing facilities. In-line, off-line, and mixed operations were evaluated. Sixteen direct or indirect egg contact surfaces were sampled in various shell egg processing facilities in the southeast United States. Samples were collected at the end of a processing day (POST) and again the next morning before operations began (PRE). Total aerobic plate counts (APCs) were obtained and Enterobacteriacae were enumerated. No significant differences ($P > 0.05$) between POST and PRE bacterial counts were found for the 16 sampling sites. In general, high APCs were found on the wall of the recirculating water tank both POST and PRE. The APCs for the rewash belt were considerably high for all plants sampled. APCs were also high for the vacuum loaders. APCs for washers and washer brushes were relatively low for most plants sampled. PRE and POST levels of plant sanitation, as determined by direct microbial plating, did not differ significantly. At this point, it is difficult to draw definitive conclusions about how rigid SSOPs should be for the shell egg processing industry.

116. Jones, F. T. and K. E. Richardson (2004). "*Salmonella* in commercially manufactured feeds."
Poult Sci **83**(3): 384-391.
http://ps.oxfordjournals.org/content/83/3/384.full.pdf
We collected 886 samples (68 feed ingredient samples, 189 dust samples, and 629 feed samples) from 3 feed mills each of which produced between 100,000 and 400,000 tons of

feed a year. Samples were collected on 3 d (Monday, Wednesday, and Friday), during 2 seasons (early spring and summer), and between 0700 and 1700 h approximately once per hour. Samples were collected from 5 locations within each mill: ingredient receiving, at the mixer, at the pellet mill, from pellet coolers, and at load-out. Temperatures were taken of the samples obtained at the pellet mill immediately following collection. All samples were analyzed for *Enterobacteriaceae* counts (EC) and *Salmonella*. The data confirm that feed ingredients and dust can be a major source of *Salmonella* contamination in feed mills. There were no differences ($P < 0.05$) in the *Salmonella* contamination rates of samples collected in spring as compared with samples collected in summer. *Salmonella* contamination rates were observed to be higher in samples collected on Friday compared with samples collected on Monday or Wednesday, an effect that may be management related. Data collected at the pellet mill clearly illustrate the uneven distribution of *Salmonella* contamination in feed as well as the need for control of dust around the pellet mill. Feed samples (both mash and pellets) contaminated with *Salmonella* contained significantly higher EC than samples not contaminated with *Salmonella*. Thus, EC may provide some indication of the likelihood of *Salmonella* contamination in feed samples.

117. Kassaify, Z. G., R. G. El Hakim, et al. (2007). "Preliminary study on the efficacy and safety of eight individual and blended disinfectants against poultry and dairy indicator organisms." Vet Ital **43**(4): 821-830.
http://www.ncbi.nlm.nih.gov/pubmed/20422561
Eight individual and blended chemical disinfectants were screened for preliminary evaluation of safety, bactericidal and virucidal effectiveness against poultry and dairy organisms. The test organisms were *Escherichia coli*, *Salmonella* Enteritidis, *Staphylococcus aureus*, *Streptococcus* spp. and *Clostridium perfringens*, in addition to avian influenza virus (AIV) and Newcastle disease virus (NDV). Viable counts of surviving bacteria were determined after 30 min contact with each disinfectant and in the presence or absence of skimmed milk, to simulate the interference of organic matter. The haemagglutination test was used to assess the survival of the test viruses in the presence of the different disinfectants after propagation in 10-day-old chick embryos. In the presence of skimmed milk, a higher concentration of most of the disinfectants examined was required to exert antimicrobial effectiveness. When used individually, quaternary ammonium showed no virucidal activity against NDV and AIV; peracetic acid was not effective against *Streptococcus* spp., *S*. Enteritidis and NDV, while iodophors showed low bactericidal and inconsistent virucidal activity. The single and blended disinfectants with high microbicidal activities included phenols (high bactericidal and virucidal activity), blends of quaternary ammonium compounds (high bactericidal activity) and blends of cresols and organic acids (high virucidal activity). This suggests the use of blends of compatible compounds for disinfection operations in poultry and dairy industries since they will target a wider range of micro-organisms. None of the disinfectants had a negative effect on the development of the different organs of chicken embryos and the iodine-based disinfectant, developed for dairy-teat dipping, also showed no adverse reactions in experimental cows.

118. Kearney, J. (2010). Food consumption trends and drivers.
http://rstb.royalsocietypublishing.org/content/365/1554/2793
A picture of food consumption (availability) trends and projections to 2050, both globally and for different regions of the world, along with the drivers largely responsible for these observed consumption trends are the subject of this review. Throughout the world, major shifts in dietary patterns are occurring, even in the consumption of basic staples towards more diversified diets. Accompanying these changes in food consumption at a global and regional level have been considerable health consequences. Populations in those countries undergoing rapid transition are experiencing nutritional transition. The diverse nature of this transition may be the result of differences in socio-demographic factors and other consumer characteristics. Among other factors including urbanization and food industry marketing, the policies of trade liberalization over the past two decades have implications for health by virtue of being a factor in facilitating the 'nutrition transition' that is associated with rising rates of obesity and chronic diseases such as cardiovascular disease and cancer. Future food policies must consider both agricultural and health sectors, thereby enabling the development of coherent and sustainable policies that will ultimately benefit agriculture, human health and the environment.

119. Keklik, N. M., A. Demirci, et al. (2010). "Pulsed UV light inactivation of *Salmonella* Enteritidis on eggshells and its effects on egg quality."
J Food Prot **73**(8): 1408-1415.
http://japr.oxfordjournals.org/content/4/4/422.full.pdf+html
The majority of *Salmonella* Enteritidis outbreaks have been related to the consumption of raw or undercooked eggs or egg-containing foods. Therefore, the U.S. Department of Agriculture mandates egg washing for all graded eggs by use of a detergent solution and sanitizer. These agencies and the egg industry have been investigating alternative decontamination techniques, which could better serve the public, minimize costs, and benefit both the public and the industry. Pulsed UV light is an emerging technology that is used to inactivate microorganisms quickly. In this study, the effectiveness of pulsed UV light was evaluated for the decontamination of eggshells. Eggs inoculated with *Salmonella* Enteritidis on the top surface at the equator were treated with pulsed UV light 1 to 30 s, at a distance of 9.5 and 14.5 cm from the UV lamp in a laboratory-scale, pulsed UV light chamber. Three eggs were used per treatment in each repetition, except for quality measurements, which involved six eggs per treatment in each repetition. A maximum log reduction of 5.3 CFU/cm2 was obtained after a 20-s treatment at 9.5 cm below the UV lamp at a total dose of 23.6+/-0.1 J/cm^2, without any visual damage to the egg. After a 30-s treatment at 9.5 and 14.5 cm, the temperature of eggshell surfaces increased by 16.3 and 13.3 degrees C, respectively. Energy usage increased up to 35.3+/-0.1 and 24.8+/-0.1 J/cm^2, after 30-s treatments at 9.5 and 14.5 cm, respectively. The effect of pulsed UV light treatments on egg quality was also evaluated. Pulsed UV-light treatments for 3, 10, and 20s at either 9.5 or 14.5 cm did not change the albumen height, eggshell strength, or cuticle presence significantly ($P<0.05$). This study demonstrated that pulsed UV light has potential to decontaminate eggshell surfaces.

120. Kinde, H., D. H. Read, et al. (1996). "Sewage effluent: likely source of *Salmonella* Enteritidis, phage type 4 infection in a commercial chicken layer flock in southern California."
Avian Dis **40**(3): 672-676.
http://www.ncbi.nlm.nih.gov/pubmed/8883800
Following the diagnosis of Salmonella enteritidis, phage type 4, infection in a commercial layer flock in southern California, effluent from a nearby sewer treatment plant was investigated as a potential source of infection. Between July 1994 and March 1995, 68 *Salmonella* isolations, comprising 27 serotypes, were made from the inflow (raw sewage) and effluent (treated sewage). Thirty-nine of 68 (57%) isolations yielded six serotypes, which consisted of *S*. Enteritidis 12% (8/68), *S*. Cerro 10% (7/68), *S*. Typhimurium 7.4% (5/68), *S*. Tennessee 7.4% (5/68), *S*. Give 7.4% (5/68), *S*. Mbandaka 7.4% (5/68), and *S*. Panama 6% (4/68). The remaining 43% (29/68) isolations were represented by 21 serotypes. Seventeen *S*. Enteritidis isolates originating from the effluent (creek water), resident feral animals (rodents, stray cats, skunks), and chickens (organs, eggs) of the affected flock were subjected to plasmid profile and restriction endonuclease analysis. Twelve of the 17 isolates had identical plasmid profile and restriction digestion patterns. Two of 17 isolates showed similar patterns but both differed from the rest; and 1 of 17 did not yield plasmids. Two other isolates were found to be different from each other and from the rest of the group.

121. Kinde, H., D. H. Read, et al. (1996). "*Salmonella* Enteritidis, phase type 4 infection in a commercial layer flock in southern California: bacteriologic and epidemiologic findings."
Avian Dis **40**(3): 665-671.
http://www.ncbi.nlm.nih.gov/pubmed/8883799
Salmonella Enteritidis, phage type 4 (SE PT4), was isolated from five of six 27-wk-old layer chickens submitted for necropsy from a flock of 43,000. Bacteriologic and epidemiologic investigations on the ranch revealed that five of the eight flocks (n = 176,000) were infected. The prevalence of SE PT4 in randomly selected healthy birds ranged from 1.7% (in caged birds) to 50% (in free-range birds) and prevalence in culled birds (kept on dirt floor houses) ranged from 14% to 42%. The estimated overall prevalence of group D *Salmonella* in eggs contaminated with group D *Salmonella* was 2.28 per 10,000. The estimated prevalence of group D *Salmonella* in eggs from caged birds in three infected houses ranged from 1.5 to 4.1 per 10,000, whereas in two houses of free-range birds, prevalence was 14.9 to 19.1 per 10,000. Three of the eight flocks on the ranch remained negative for *Salmonella* between May 1994 and December 1995 or until removed from the ranch. *Salmonella* Enteritidis PT4 was also isolated from 12.5% (6 of 48) of mice; 57% (four of seven) of cats; and two of two skunks

tested. Environmental drag swabs and well water samples yielded multiple serotypes of *Salmonella* (23/180 and 5/14, respectively) but not *S.* Enteritidis.

122. Kirk, M., L. Ford, et al. (2014). "Foodborne illness, Australia, circa 2000 and circa 2010." Emerg Infect Dis **20**(11): 1857-1864. http://www.health.gov.au/internet/main/publishing.nsf/Content/E829FA59A59677C0CA257D6A007D2C97/$File/Foodborne-Illness-Australia-circa-2010.pdf
Foodborne disease is a major public health problem worldwide. To examine changes in foodborne illness in Australia, we estimated the incidence, hospitalizations, and deaths attributed to contaminated food circa 2010 and recalculated estimates from circa 2000. Approximately 25% of gastroenteritis cases were caused by contaminated food; to account for uncertainty we used simulation techniques to estimate 90% credible intervals. We estimate that circa 2010, 4.1 million foodborne gastroenteritis cases occurred, and circa 2000, 4.3 million cases occurred. Circa 2010, contaminated food was estimated to be responsible for 30,840 gastroenteritis-associated hospitalizations, 76 associated deaths, and 5,140 nongastrointestinal illnesses. Cases of salmonellosis and campylobacteriosis increased from 2000 to 2010 and were the leading causes of gastroenteritis-associated hospitalizations; *Listeria monocytogenes* and nontyphoidal *Salmonella* spp. infections were the leading causes of death. Although the overall incidence of foodborne illnesses declined over time in Australia, cases of foodborne gastroenteritis are still common.

123. Klowden, M. J. and B. Greenberg (1976). "*Salmonella* in the American cockroach: evaluation of vector potential through dosed feeding experiments." J Hyg (Lond) **77**(1): 105-111. http://www.ncbi.nlm.nih.gov/pubmed/789761
Restrained American cockroaches, Periplaneta americana L., were fed graded doses of *Salmonella* Typhimurium ranging from 1-6 X 10(3) to 2-0 X 10(6), and their faeces assayed daily for the pathogen. Only 4 specimens out of 117 demonstrated multiplication of salmonellas, which was unrelated to size of input. When data of persistence regardless of actual numbers were expressed as percentage *Salmonella*-positive faecal-days, and these transformed to probits, a graph of percentage-positive faecal-days versus log dose allowed a calculation of the CD 50, or contaminative dose required for 50% of the faecal-days to be infective. The CD 50 for this cockroach species was 1-4 X 10(6) *Salmonella*.

124. Kogut, M. H., K. J. Genovese, et al. (1999). "Effect of induced molting on heterophil function in White Leghorn hens." Avian Dis **43**(3): 538-548. http://www.ncbi.nlm.nih.gov/pubmed/10494425
This study was undertaken to determine the effects of induced molt on basal functional activities of heterophils from aging hens. For this purpose, heterophils from both molted and unmolted hens were examined by in vitro bioassays for functional responsiveness and efficiency. We evaluated the ability of the heterophils to migrate to chemotactic stimuli, phagocytize opsonized and nonopsonized *Salmonella* Enteritidis (SE), and generate an oxidative burst in response to inflammatory agonists. A significant ($P < 0.001$) heterophilia was found in the molted hens within 2 days after feed withdrawal and remained throughout the length of the experimental feed withdrawal period. No significant differences were found in the random migration of heterophils from either group. The chemotactic movement of heterophils from molted hens was not affected until 8 days after feed withdrawal when compared with heterophil chemotaxis from unmolted hens. A significant decrease in chemotaxis by the heterophils from molted hens was observed days 8-12 after feed withdrawal ($P < 0.05$). Significantly ($P < 0.05$) fewer heterophils from molted hens were able to phagocytize opsonized (59% vs. 38%) and nonopsonized (26% vs. 15%) SE within 2 days after feed withdrawal. Likewise, significantly ($P < 0.05$) fewer bacteria were phagocytized per heterophil from the molted hens when compared with the number of bacteria per heterophil from the unmolted hens. The oxidative burst of heterophils stimulated by either opsonized zymosan A or phorbol myristate acetate of heterophils from molted hens was significantly ($P < 0.05$) reduced when compared with that generated by heterophils from the unmolted hens. These results indicate that feed withdrawal to induce molt alters the number and function of peripheral blood heterophils. This decreased efficiency of heterophil functional activity appears to play a role in the increased susceptibility of molting hens to SE infections.

125. Koyuncu, S., M. G. Andersson, et al. (2013). "Organic acids for control of *Salmonella* in different feed materials."
BMC Vet Res **9**: 81.
http://www.biomedcentral.com/1746-6148/9/81
BACKGROUND: *Salmonella* control in animal feed is important in order to protect animal and public health. Organic acids is one of the control measures used for treatment of *Salmonella* contaminated feed or feed ingredients. In the present study, the efficacy of formic acid (FA) and different blends of FA, propionic acid (PA) and sodium formate (SF) was investigated. Four *Salmonella* strains isolated from feed were assayed for their acid tolerance. Also, the effect of lower temperatures (5 degrees C and 15 degrees C) compared to room temperature was investigated in rape seed and soybean meal.
RESULTS: The efficacy of acid treatments varied significantly between different feed materials. The strongest reduction was seen in pelleted and compound mash feed (2.5 log10 reduction) followed by rapeseed meal (1 log10 reduction) after 5 days exposure. However, in soybean meal the acid effects were limited (less than 0.5 log10 reduction) even after several weeks' exposure. In all experiments the survival curves showed a concave shape, with a fast initial death phase followed by reduction at a slower rate during the remaining time of the experiment.No difference in *Salmonella* reduction was observed between FA and a blend of FA and PA, whereas a commercial blend of FA and SF (Amasil) was slightly more efficacious (0.5-1 log10 reduction) than a blend of FA and PA (Luprocid) in compound mash feed. The *Salmonella* Infantis strain was found to be the most acid tolerant strain followed by, *S*. Putten, *S.* Senftenberg and *S*. Typhimurium. The tolerance of the *S.* Infantis strain compared with the *S*. Typhimurium strain was statistically significant ($p<0.05$). The lethal effect of FA on the *S*. Typhimurium strain and the *S.* Infantis strain was lower at 5 degrees C and 15 degrees C compared to room temperatures.
CONCLUSIONS: Acid treatment of *Salmonella* in feed is a matter of reducing the number of viable bacterial cells rather than eliminating the organism. Recommendations on the use of acids for controlling *Salmonella* in feed should take into account the relative efficacy of acid treatment in different feed materials, the variation in acid tolerance between different *Salmonella* strains, and the treatment temperature.

126. Koyuncu, S. and P. Haggblom (2009). "A comparative study of cultural methods for the detection of *Salmonella* in feed and feed ingredients."
BMC Vet Res **5**: 6.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2642806/
BACKGROUND: Animal feed as a source of infection to food producing animals is much debated. In order to increase our present knowledge about possible feed transmission it is important to know that the present isolation methods for *Salmonella* are reliable also for feed materials. In a comparative study the ability of the standard method used for isolation of *Salmonella* in feed in the Nordic countries, the NMKL71 method (Nordic Committee on Food Analysis) was compared to the Modified Semisolid Rappaport Vassiliadis method (MSRV) and the international standard method (EN ISO 6579:2002). Five different feed materials were investigated, namely wheat grain, soybean meal, rape seed meal, palm kernel meal, pellets of pig feed and also scrapings from a feed mill elevator. Four different levels of the *Salmonella* serotypes *S*. Typhimurium, *S*. Cubana and *S*. Yoruba were added to each feed material, respectively. For all methods pre-enrichment in Buffered Peptone Water (BPW) were carried out followed by enrichments in the different selective media and finally plating on selective agar media.
RESULTS: The results obtained with all three methods showed no differences in detection levels, with an accuracy and sensitivity of 65% and 56%, respectively. However, Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn), performed less well due to many false-negative results on Brilliant Green agar (BGA) plates. Compared to other feed materials palm kernel meal showed a higher detection level with all serotypes and methods tested.
CONCLUSION: The results of this study showed that the accuracy, sensitivity and specificity of the investigated cultural methods were equivalent. However, the detection levels for different feed and feed ingredients varied considerably.

127. Lapuz, R. R., D. V. Umali, et al. (2012). "Comparison of the prevalence of *Salmonella* infection in layer hens from commercial layer farms with high and low rodent densities."

Avian Dis **56**(1): 29-34.
http://www.ncbi.nlm.nih.gov/pubmed/22545525
A comparison on the prevalence of *Salmonella* infection in layer hens from commercial layer farms with high and low rodent densities was investigated. Out of 280 laying hens sampled from three commercial layer farms with high rodent densities, *Salmonella enterica* subsp. enterica serovar Enteritidis (*Salmonella* Enteritidis) was isolated from 20 (7.14%) hens and *Salmonella enterica* subsp. enterica serovar Infantis (*Salmonella* Infantis) from three (1.07%) hens. In contrast, layer hens sampled from four commercial layer farms with low rodent densities were negative for any salmonellae. Significant differences ($P < 0.05$) in the isolation rates of *Salmonella* from various organs of infected layer hens were also noted. For *Salmonella* Enteritidis, liver (55.0%) and the oviduct (55.0%) had the highest isolation rates while all Salmonella Infantis isolates were from the oviduct. Pulsed field gel electrophoresis (PFGE) analysis of BlnI-digested chromosomal DNA of *Salmonella* Enteritidis isolated from layer hens and rodents showed similar patterns. PFGE analysis of *Salmonella* Infantis isolated from layer hens, rodents, eggs, and the environment yielded identical patterns. In this study, the significantly higher prevalence rate ($P < 0.05$) of *Salmonella* Enteritidis and *Salmonella* Infantis in layer hens from high rodent density farms could be attributed to the high rodent population density. The persistent *Salmonella* Enteritidis and *Salmonella* Infantis infection inside layer houses may have been amplified by the increasing numbers in the rodent population over the years, which increased the opportunity for environment-rodent-chicken interaction and the transmission of salmonellae to chickens. Monitoring of salmonellae from rodents inside poultry premises is recommended to be an effective additional tool in the assessment of the *Salmonella* status of layer flocks.

128. Lublin, A. and S. Sela (2008). "The impact of temperature during the storage of table eggs on the viability of *Salmonella enterica* serovars Enteritidis and Virchow in the Eggs."
Poult Sci **87**(11): 2208-2214.
http://ps.oxfordjournals.org/content/87/11/2208.long
Salmonellosis is a foodborne infection of major economic importance. Contamination of table eggs with *Salmonella*, especially *Salmonella* enterica serovar Enteritidis, is a major health concern worldwide. Recently, *S. enterica* serovar Virchow has emerged as a major pathogen in Israel, where it is among the 3 most prevalent serovars found in poultry and the second most prevalent serovar isolated from individuals with salmonellosis. Although there is ample knowledge regarding the role of *S. enterica* serovar Enteritidis in contamination of eggs, virtually nothing is known regarding the possible association of *S. enterica* serovar Virchow with table eggs. Therefore, our goal was to examine the capability of serovar Virchow to contaminate chicken eggs. Commercial table eggs were inoculated independently with serovar Enteritidis and with serovar Virchow cells at a concentration of 10(5) cfu/egg, either on the shell surface or by injection into the yolk. The numbers of live Salmonella cells on the shell and within the egg were determined at various time points. At both low (6 degrees C) and room temperatures (25 degrees C), *S. enterica* serovar Virchow was not detected on the eggshell after 2 wk, whereas *S. enterica* serovar Enteritidis could be detected only sporadically at 25 degrees C. In contrast, within the eggs, *S. enterica* serovar Virchow survived for up to 6 wk at 6 degrees C, and it multiplied up to 10(9) cfu/mL of egg content from 2 to 8 wk postinoculation at 25 degrees C. In comparison, *S. enterica* serovar Enteritidis survived within the eggs up to 8 wk at 6 degrees C and at 25 degrees C. Our results suggest that in cold storage, serovar Virchow is able to persist for long periods (6 wk), and at room temperature, these bacteria can multiply within eggs and reach high concentrations. Therefore, eggs might be considered potential vectors for transmitting *S. enterica* serovar Virchow into the food chain.

129. Maciorowski, K. G., P. Herrera, et al. (2006). "Cultural and immunological detection methods for *Salmonella* spp. in animal feeds - A review."
Vet Res Commun **30**(2): 127-137.
http://link.springer.com/article/10.1007%2Fs11259-006-3221-8
Food-borne salmonellosis continues to be a major public health concern, and contamination with *Salmonella* spp. in pre-harvest animal production is considered a primary contributor to this problem. Animal feeds can easily become contaminated during primary production, feed mixing and processing as well as during feeding. Consequently, monitoring and surveillance of feeds and feed ingredients for *Salmonella* spp. contamination may be useful or necessary

in the prevention and control of this organism. Cultural and immunological detection methods for salmonellae have been used or suggested as possible approaches for use in animal feeds. Cultural methods remain advantageous owing to their ability to detect viable bacterial cells, while immunological methods have the capability of detecting nonculturable bacterial cells. Advancements and improvements in both methodologies offer opportunities for eventual routine use of these detection technologies in animal feed assays.

130. Mackey, B. M. and C. Derrick (1990). "Heat shock protein synthesis and thermotolerance in *Salmonella* Typhimurium."
J Appl Bacteriol **69**(3): 373-383.
http://www.ncbi.nlm.nih.gov/pubmed/2246142
The resistance of stationary phase *Salmonella* Typhimurium to heating at 55 degrees C was greater in cells grown in nutritionally rich than in minimal media, but in all media tested resistance was enhanced by exposing cells to a primary heat shock at 48 degrees C. Chloramphenicol reduced the acquisition of thermotolerance in all media but did not completely prevent it in any. The onset of thermotolerance was accompanied by increased synthesis of major heat shock proteins of molecular weight about 83, 72, 64 and 25 kDa. When cells were shifted from 48 degrees C to 37 degrees C, however, thermotolerance was rapidly lost with no corresponding decrease in the levels of these proteins. There is thus no direct relationship between thermotolerance and the cellular content of the major heat shock proteins. One minor protein of molecular weight about 34 kDa disappeared rapidly following a temperature down-shift. Its presence in the cell was thus correlated with the thermotolerant state.

131. Mackey, B. M. and C. M. Derrick (1987). "Changes in the heat resistance of *Salmonella* Typhimurium during heating at rising temperatures."
Lett Appl Microbiol **4**(1): 13-16.
http://dx.doi.org/10.1111/j.1472-765X.1987.tb01571.x
The heat resistance of *Salmonella* Typhimurium, measured as survival following a standard heat challenge at 55°C for 25 min, increased progressively as cells were heated up at linearly rising temperatures. The amount by which heat resistance increased depended on the rate of temperature rise; the slower the temperature rise, the greater the increase in resistance.

132. Maguire, H., P. Pharoah, et al. "Hospital outbreak of *Salmonella* Virchow possibly associated with a food handler."
J Hosp Infect **44**(4): 261-266
A foodborne outbreak of salmonella infection at a private hospital in London in 1994 was found to be associated with eating turkey sandwiches prepared by a food handler. One patient, nine staff, and a foodhandler's baby were confirmed to have *Salmonella enterica* serotype virchow, phage type 26 infection. The attack rate was estimated to be 5% among the approximately 200 patients and staff at risk. A food handler reportedly became ill days after, but her baby days before, the first hospital case. Although it appeared to be a single outbreak, antibiogram analysis, supplemented by plasmid profile typing, demonstrated that there were two strains of S. virchow involved, one with resistance to sulphonamides and trimethoprim and a second sensitive to these antimicrobial drugs. Mother and child had different strains. The investigation demonstrated the importance of full phenotypic characterization of putative outbreak strains including antimicrobial susceptibility testing.Outbreaks of foodborne infection in hospitals are preventable and are associated with high attack rates and disruption of services. There is a need for good infection control policies and training of all staff involved in patient care as well as in catering services. Consultants in Communicable Disease (CCDCs) should include private hospitals in their outbreak control plans. Good working relations between Infection Control Doctors (ICDs) in the private health sector and their local CCDCs are important if outbreaks are to be properly investigated.

133. Majtan, V. and L. Majtanova (1997). "Antibacterial efficacy of new commercially manufactured disinfectant substances against *Salmonella* Typhimurium."
J Basic Microbiol **37**(1): 41-44.
http://www.ncbi.nlm.nih.gov/pubmed/9090125
The antibacterial effect of 19 new commercially manufactured disinfectant substances on a *Salmonella* Typhimurium strain was studied. The substances tested represent 9 quaternary

ammonium salts (QAT) and 11 combinated QAT with other ingredients. The antimicrobial efficacy was characterized by influencing the growth and reproduction of bacterial cells expressed either by MIC and ED50 values (ED50 values represent concentration of substance in micrograms/ml which cause inhibition of growth by 50%), as well as by the inhibition of incorporation rate of [14C]leucine. The disinfectants are divided into three groups according to their efficacy. The first group comprises substances with strong inhibitory effect (MIC 0.04-0.19 microgram/ml) such as Neoquat S, Antibacteric P, Divoquat forte, Sokrena and Diesin forte (sole from the group belonging to multicomponent substances). QAT except Antibacteric Pinterfere with energy metabolism (R values approximately 1). The second group represents substances with good antibacterial efficacy (MIC values up 1.56 micrograms/ml), and the third group substances with good antibacterial efficacy (MIC values up 1.56 micrograms/ml), and the third group substances with MIC values up 12.5 micrograms/ml. Cutasept G was found ineffective also in the concentration 100 micrograms/ml. The method of inhibition of [14C] precursors is suitable as one from possible criterion in evaluation of antibacterial efficacy of various synthetic substances.

134. Marin, C., S. Balasch, et al. (2011). "Sources of *Salmonella* contamination during broiler production in Eastern Spain."
Prev Vet Med **98**(1): 39-45.
http://www.ncbi.nlm.nih.gov/pubmed/21035883
Prevention of *Salmonella* contamination of poultry products requires detailed knowledge of the main sources associated with its presence in the production system. The aims of this study were to determine the main sources of *Salmonella* contamination in broiler production during growing, to assess the risk factors for *Salmonella* contamination at the end of the rearing period and to determine the main serovars involved in broiler production systems in Eastern Spain. A total of 65 different broiler houses from different farms were sampled. Each house was sampled at different times during the rearing period. First, when the previous flock was taken to the slaughterhouse, samples of dust, surfaces and previous flock faeces were collected. After cleaning and disinfection (C&D), samples of dust and surfaces were also taken. On the first day of rearing, samples of water, bedding, farming boots, meconiums, delivery-box liners and feed were collected. During rearing, feed samples were taken directly from the truck and from feeders. On slaughter day, samples of dust, surfaces, water, feed and faeces were also collected. Finally, two days after slaughter, carriers (rodents, flies and beetles) were trapped. All samples collected were analysed according to ISO 6579:2002 (Annex D) and positive samples were serotyped in accordance with Kauffman-White-Le-Minor technique. Our results showed that all different types of samples collected were contaminated with *Salmonella* (prevalence ranged between 1.5% and 38.6%). The most contaminated samples related with poultry production were: delivery-box liners (32.0%), faeces samples (31.2%), dust samples (25.0%), farming boots (19.7%) and feed from feeders (16.0%). However, the most important risk factors for Salmonella contamination of the flocks at the end of the rearing period were *Salmonella* status of the house after cleaning and disinfection, *Salmonella* status of day-old chick flocks and feed from feeders. Twenty-one different serovars were isolated from the samples analysed. The most prevalent were in decreasing order: *Salmonella* Enteritidis (52.9%), *S*. Hadar (17.8%), *S*. Virchow (8.9%) and *S*. Ohio (5.4%). The study suggested that there are many sources for *Salmonella* contamination and persistence in broiler production. Hence, the whole production chain needs to be controlled to eradicate the bacteria from primary production.

135. Marin, C. and M. Lainez (2009). "*Salmonella* detection in feces during broiler rearing and after live transport to the slaughterhouse."
Poult Sci **88**(9): 1999-2005.
http://ps.oxfordjournals.org/content/88/9/1999.full.pdf
Eggs and poultry meat are a common source of human salmonellosis. Contamination of poultry or poultry meat may occur throughout the production chain. Nevertheless, in Spain, noncontaminated broiler meat may be sold for human consumption from 2011. The aims of this study were as follows:
(i) assess *Salmonella* detection from feces samples during the rearing and
(ii) assess the influence of live transport to the slaughterhouse on *Salmonella* detection. During this study, 65 flocks were sampled at weekly intervals from the first day of rearing until slaughter. Samples of feces were taken from the litter using 5 pairs of cellulose sock swabs

attached to boots and applied over the length of the house. To assess *Salmonella* detection rates before and after live transport to the slaughterhouse, feces samples were collected. Before loading, feces with 5 pairs of cellulose sock swabs were taken as described above. After transport, 2 pooled feces samples were taken directly from the truck (200 to 300 g each). All samples were analyzed in accordance with ISO 6579:2002 (Annex D). Results showed that regardless of whether broiler flocks arrived at the farm already shedding the bacteria in feces or they were infected during rearing, both groups described the same detection pattern, with the highest detection in feces at 14th day of rearing (50.5 and 34.5%, respectively). Moreover, *Salmonella* Enteritidis was the most prevalent serotype isolated during rearing (66.7%), followed by *Salmonella* Virchow (13.7%), *Salmonella* Hadar (9.4%), and *Salmonella* Ohio (2.8%). On the other hand, before loading and after transport to the slaughterhouse, 15.4 and 41.2% of feces samples collected were determined positive, respectively. In addition, a change in the serotype pattern was also observed. *Salmonella* Enteritidis remains the most prevalent serotype isolated (54.5%). *Salmonella* Hadar doubled the excretion rates (39.3%), and *Salmonella* Virchow and *Salmonella* Ohio were not isolated after transport.

136. Mashaly, M. M., G. L. Hendricks, 3rd, et al. (2004). "Effect of heat stress on production parameters and immune responses of commercial laying hens."
Poult Sci **83**(6): 889-894.
http://www.ncbi.nlm.nih.gov/pubmed/15206614
The present study was conducted to determine the adverse effects of high temperature and humidity not only on live performance and egg quality but also on immune function in commercial laying hens. One hundred eighty 31-wk-old laying hens at peak production were used in this study. Hens were housed in cages (15 cages of 4 birds/cage) in each of 3 environmental chambers and received 1 of 3 treatments. The 3 treatments were control (average temperature and relative humidity), cyclic (daily cyclic temperature and humidity), and heat stress (constant heat and humidity) for 5 wk. Different production and immune parameters were measured. Body weight and feed consumption were significantly reduced in hens in the heat stress group. Egg production, egg weight, shell weight, shell thickness, and specific gravity were significantly inhibited among hens in the heat stress group. Likewise, total white blood cell (WBC) counts and antibody production were significantly inhibited in hens in the heat stress group. In addition, mortality was higher in the heat stress group compared to the cyclic and control groups. Even though T- and B-lymphocyte activities were not significantly affected by any of the treatments, lymphocytes from hens in the heat stress group had the least activity at 1 wk following treatment. These results indicate that heat stress not only adversely affects production performance but also inhibits immune function.

137. Mastroeni, P., J. A. Chabalgoity, et al. (2001). "*Salmonella*: immune responses and vaccines."
Vet J **161**(2): 132-164.
http://www.ncbi.nlm.nih.gov/pubmed/11243685
Salmonella infections are a serious medical and veterinary problem world-wide and cause concern in the food industry. Vaccination is an effective tool for the prevention of Salmonella infections. Host resistance to *Salmonella* relies initially on the production of inflammatory cytokines leading to the infiltration of activated inflammatory cells in the tissues. Thereafter T- and B-cell dependent specific immunity develops allowing the clearance of *Salmonella* microorganisms from the tissues and the establishment of long-lasting acquired immunity to re-infection. The increased resistance that develops after primary infection/ vaccination requires T-cells cytokines such as IFNgamma TNFalpha and IL12 in addition to opsonising antibody. However for reasons that are not fully understood seroconversion and/or the presence of detectable T-cell memory do not always correlate with the development of acquired resistance to infection.Whole-cell killed vaccines and subunit vaccines are used in the prevention of *Salmonella* infection in animals and in humans with variable results. A number of early live *Salmonella* vaccines derived empirically by chemical or u.v. mutagenesis proved to be immunogenic and protective and are still in use despite the need for repeated parenteral administration. Recent progress in the knowledge of the genetics of *Salmonella* virulence and modern recombinant DNA technology offers the possibility to introduce multiple defined attenuating and irreversible mutations into the bacterial genome. This has recently allowed the development of *Salmonella* strains devoid of significant side effects but still capable of inducing solid immunity after single oral administration. Live attenuated *Salmonella*

vaccines have been used for the expression of heterologous antigens/proteins that can be successfully delivered to the immune system. Furthermore *Salmonella* can transfer plasmids encoding foreign antigens under the control of eukaryotic promoters (DNA vaccines) to antigen-presenting cells resulting in targeted delivery of DNA vaccines to these cells. Despite the great recent advances in the development of *Salmonella* vaccines a large proportion of the work has been conducted in laboratory rodents and more research in other animal species is required.

138. Matches, J. R. and J. Liston (1968). "Low Temperature Growth of *Salmonella*."
J Food Sci **33**(6): 641-645.
http://dx.doi.org/10.1111/j.1365-2621.1968.tb09092.x
Salmonellae, normally grown at 3.5–37°C, can grow at much lower temperatures. Experimentally, minimum growth temperatbres were obtained when salmonellae were grown on the surface of agar in a temperature-gradient incubator over a temperature range of 1.1 to 12.3°C. These minimum temperatures, as determined by visible growth for 7 serotypes, ranged from 5.5 to 6.8°C. The pattern of survival or growth of *S*. derby, *S.* heidelberg, and *S.* typhimurium was followed by inoculating the organisms into tubes of broth and incubating the tubes in a polythermostat over a temperature range of 1.1 to 12.3°C. Minimum growth temperature obtained for *S.* heidelberg after 19 days' incubation was 5.3°C. The minimum growth temperature for the same length of time for *S*. typhimurium and S. derby were 6.2 and 6.9°C respectively. The results indicate a growth temperature shift during extended incubation of *Salmonella* at low temperatures. This phenomenon and the low temperature. growth capability of *Salmonella* could be significant in foods stored for long periods of time at temperatures above 5°C.

139. Mattick, K., K. Durham, et al. (2003). "The survival of foodborne pathogens during domestic washing-up and subsequent transfer onto washing-up sponges, kitchen surfaces and food."
Int J Food Microbiol **85**(3): 213-226.
http://www.ncbi.nlm.nih.gov/pubmed/12878380
In this study, the survival of *Salmonella*, *Campylobacter* and *Escherichia coli* O157: H7, when exposed to a range of constant temperatures (47-60 degrees C), in hard or soft water, in the presence/absence of detergent (0-0.3%) and organic matter, and during drying, was investigated. Further experiments used a washing-up process simulation, where soiled dishes contaminated with bacteria were washed in a bowl of warm water containing detergent. In addition, this study considered the risk of bacterial transfer onto (1) sterile dishes and sponges via contaminated water, (2) kitchen surfaces wiped with a contaminated sponge, (3) items placed in direct contact with a contaminated kitchen surface, (4) food placed on a contaminated dish or (5) dishes from contaminated food. A proportion of dishes remained contaminated with all pathogen types after a typical washing-up. Water hardness did not appear to affect survival. *E. coli*, and to a lesser extent *Salmonella*, survived towel- or air-drying on dishes and after towel-drying the cloth became contaminated on every occasion, regardless of the test organism. A proportion of sterile dishes washed after contaminated dishes became contaminated with pathogens but transfer from dishes onto food was rare. Washing-up sponges frequently became contaminated with pathogens. The results of this study highlight the potential for survival and cross contamination of food borne pathogens in the kitchen environment.

140. Mattick, K., K. Durham, et al. (2003). "The microbiological quality of washing-up water and the environment in domestic and commercial kitchens."
J Appl Microbiol **94**(5): 842-848.
http://www.ncbi.nlm.nih.gov/pubmed/12694449
AIMS: To determine the microbiological quality of washing-up water and the environment in domestic and commercial kitchens.
METHODS AND RESULTS: Chicken meals were prepared by people without food safety training in their own kitchen (n = 52) or by trained staff in a commercial kitchen (n = 10). Study participants then washed-up, cleaned the kitchen and completed a food hygiene questionnaire. The temperature and microbiological quality of the washing-up water, and the presence of pathogens in dishcloths, tea towels and other kitchen samples was determined. Of the raw chickens used in meal preparation, 96 and 13% were naturally contaminated with *Campylobacter* or *Salmonella* spp., respectively. In domestic kitchens, two of 45 sponges,

dishcloths or scourers and one of 32 hand- or tea towels were contaminated with *Campylobacter* after washing-up and cleaning but none of the tap or sink swabs yielded pathogens. The mean washing-up water temperature in the domestic kitchens was 40.7 degrees C, whereas in the commercial kitchen it was 44.7 degrees C (P = 0.04). Study participants who used hotter water (>/=40 degrees C) had lower levels of bacteria in their washing-up water. The aerobic plate counts of the washing-up water samples in domestic homes were usually between 10^5 and 106 CFU ml^{-1} but those associated with the commercial kitchen were consistently lower (P = 0.01). Despite this, *Campylobacter* was detected in one of 10 washing-up water samples from the commercial kitchen but in none of the samples from domestic kitchens.
CONCLUSIONS: Pathogenic microorganisms can be recovered relatively frequently from the kitchen environment.
SIGNIFICANCE AND IMPACT OF STUDY: By identifying factors that affect the number of microorganisms in washing-up water and the kitchen environment, evidence-based recommendations on implementing domestic food hygiene can be made.

141. McAuley, C. M., L. L. Duffy, et al. (2015). "*Salmonella* Typhimurium and *Salmonella* Sofia: growth in and persistence on eggs under production and retail conditions."
BioMed Res Int **2015**:8
http://www.hindawi.com/journals/bmri/aa/914987/
Salmonellosis in Australia has been linked to eggs and egg products with specific serotypes associated with outbreaks. We compared attachment to and survival on egg shells and growth in eggs of two *Salmonella* serotypes, an egg outbreak associated *Salmonella* Typhimurium and a non-egg-associated *Salmonella enterica* ssp. II 1,4,12,27:b:[e,n,x] (*S*. Sofia). Experiments were conducted at combinations of 4, 15, 22, 37 and 42°C. No significant differences occurred between the serotypes in maximum growth rates, which were significantly greater (P<0.001) in egg yolk (0.427 log10 CFU/mL/h) compared to whole egg (0.312 log10 CFU/mL/h) and egg white (0.029 log10 CFU/mL/h). Attachment to egg shells varied by time (1 or 20 min) and temperature (4, 22 and 42°C), with *S*. Typhimurium isolates attaching at higher levels (P<0.05) than *S*. Sofia after 1 min at 4°C and *S.* Typhimurium ATCC 14028 attaching at higher (P<0.05) levels at 22°C. Survival on egg shells was not significantly different across isolates. *Salmonella* serotypes behaved similarly regarding growth in egg contents, attachment to egg shells and survival on eggs, indicating that other factors more likely contributed to reasons for *S*. Typhimurium being implicated in multiple egg-associated outbreaks.

142. McQuestin, O. J., M. T. Musgrove, et al. (2010). "Kinetics of growth and inactivation of *Salmonella enterica* serotype Typhimurium DT104 in pasteurised liquid egg products."
Food Microbiol **27**(3): 396-402.
http://www.ncbi.nlm.nih.gov/pubmed/20227605
The potential impact of post-pasteurisation contamination of liquid egg products with the multi-antibiotic resistant pathogen *Salmonella enterica* serotype Typhimurium definitive type 104 (DT104) was assessed by determining the viability of this bacterium in whole egg, albumen and 10% w/w sugared and salted yolk incubated at 4-42 degrees C. Results indicated that populations of *S*. Typhimurium DT104 were slowly inactivated in all four products when stored at 4 degrees C. However, based on the typical shelf-lives of cold-stored liquid egg, less than 0.6 log-kill would be achieved in those products prior to their use. Incubation at temperatures pertaining to abuse situations (10, 15, 20 and 25 degrees C) revealed an increasing potential for growth of *S.* Typhimurium DT104 in whole egg, albumen and sugared yolk, as indicated by trends in growth rate, lag duration and maximum population density. At even higher temperatures (30, 37 and 42 degrees C), growth rates of *S*. Typhimurium DT104 in whole egg and sugared yolk continued to increase. The same was true for *S*. Typhimurium DT104 in albumen except that growth was not observed at 42 degrees C and instead populations were inactivated within 30 h. At no temperature tested was *S*. Typhimurium DT104 able to grow in salted yolk. The influence of these growth and inactivation patterns on the risk of salmonellosis in relation to product type and storage temperature is discussed.

143. McWhorter, A. R. and K. K. Chousalkar (2015). "Comparative phenotypic and genotypic virulence of *Salmonella* strains isolated from Australian layer farms."

Front Microbiol **6**: 12.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4304256/
There are over 2500 *Salmonella enterica* serovars that circulate globally. Of these, serovars those classified into subspecies I are the most common cause of human salmonellosis. Many subspecies I *Salmonella* serovars are routinely isolated from egg farm environments but are not frequently associated with causing disease in humans. In this study, virulence profiles were generated for 10 strains of *Salmonella enterica* isolated directly from egg farm environments to investigate their potential public health risk. Three virulence parameters were assessed including in vitro invasion, in vivo pathogenicity and characterization of genomic variation within five specific pathogenicity islands. These 10 *Salmonella* strains exhibited significant differences in invasion into the human intestinal epithelial cell line, Caco2. Low, moderate, and high invasion patterns were observed and the degree of invasion was dependent on bacterial growth in a nutritive environment. Interestingly, two *Salmonella* strains, *S*. Adelaide and *S*. Bredeney had consistently low invasion. The *S*. Typhimurium definitive types and *S*. Virchow exhibited the greatest cell invasion following growth in Luria Bertani broth. Only the *S*. Typhimurium strains caused disease in BALB/c mice, yet the majority of serovars were consistently detected in feces over the 21 day experiment. Genomic comparison of the five specific pathogenicity islands has shown that variation in virulence is likely multifactorial. Sequence variability was observed primarily in strains with low virulence. In particular, genes involved in forming the structures of the SPI-1 and SPI-2 type 3 secretion systems as well as multiple effector proteins were among the most variable. This variability suggest that serovars with low virulence are likely to have both invasion and within host replication defects that ultimately limit their pathogenicity.

144. Medus, C., K. E. Smith, et al. (2006). "*Salmonella* outbreaks in restaurants in Minnesota, 1995 through 2003: evaluation of the role of infected foodworkers."
J Food Prot **69**(8): 1870-1878.
http://www.ncbi.nlm.nih.gov/pubmed/16924912
The 23 restaurant-associated salmonellosis outbreaks that occurred in Minnesota from 1995 through 2003 were reviewed to characterize the role of infected foodworkers. The median duration of the outbreaks was 21 days (range, 1 to 517 days). The median number of culture-confirmed patron cases per outbreak was seven (range, 1 to 36 cases). The median incubation for patron cases ranged from 9 h to 5.9 days. A specific food vehicle was implicated in four outbreaks and suspected in five. *Salmonella* of the same serotype and pulsed-field gel electrophoresis subtype as that found in patrons was recovered from foodworkers in 19 outbreaks. Overall, 12% (129 of 1,033) of foodworkers tested positive for *Salmonella*. Sixty-four (53%) of 121 *Salmonella*-positive foodworkers reported not having had a recent gastrointestinal illness. Overall, the median duration of *Salmonella* shedding was 16 days. Among foodworkers who reported gastrointestinal illness, the median shedding duration was 30 days as compared with 3 days for asymptomatic foodworkers. Positive environmental samples were recovered in 4 (33%) of 12 outbreaks. No specific food vehicle was identified in any outbreaks associated with *Salmonella*-positive environmental samples. The median duration of outbreaks with positive environmental samples (187 days) was significantly longer than the median duration of outbreaks with negative environmental results (26 days, P = 0.03). A higher proportion of *Salmonella*-positive foodworkers (22 versus 8%) was identified in outbreaks with positive environmental samples. *Salmonella* outbreaks in restaurants are frequently prolonged yet produce a small number of confirmed patron cases. Prolonged outbreak durations suggest a persistent reservoir of contamination. Infected foodworkers likely serve as an important source for *Salmonella* transmission. Therefore, assessment of foodworker infection is essential for controlling restaurant outbreaks.

145. Meerburg, B. G. and A. Kijlstra (2007). "Role of rodents in transmission of *Salmonella* and Campylobacter."
J Sci Food Agr **87**(15): 2774-2781.
http://dx.doi.org/10.1002/jsfa.3004
Salmonella and *Campylobacter* are generally regarded as the most important food-borne pathogens in the world. Reduction or elimination of these pathogens in the first part of the food chain (on the farm) is important to prevent disease among consumers of animal products. In organic farming, elimination becomes more difficult, as food animals are allowed outdoors and have easy access to potential sources of hazardous pathogens. Whilst rodents

are often associated by organic farmers with infrastructural damage and eating or spoiling of stored feed and products, their zoonotic risks are frequently underestimated. They can amplify the number of pathogens in the environment and transfer them to food animals. Thus organic farmers should be aware of the need for rodent control from a food safety perspective. Preferably, rodent control should form an integral part of a total package of hygiene measures to prevent transfer of food-borne pathogens. These should also include e.g. control of wild birds and flies and obligatory disinfection of boots/clothes and equipment for farm workers and visitors.

146. Membre, J.-M., V. Majchrzak, et al. (1997). "Effects of Temperature, pH, Glucose, and Citric Acid on the Inactivation of *Salmonella* Typhimurium in Reduced Calorie Mayonnaise." J Food Prot **60**(12): 1497-1501. http://www.ingentaconnect.com/content/iafp/jfp/1997/00000060/00000012/art00005
A *Salmonella* Typhimurium strain was inoculated in reduced calorie mayonnaise. A central composite design was implemented to assess the effects of temperature (15 to 35°C), pH (4.5 to 6.5), glucose (1 to 4% [wt/vol]), and citric acid (0.05 to 0.1 % [wt/vol]) on the inactivation of *Salmonella*. Whatever the conditions, an inhibition of the strain was obtained, but only after a long period of time, from 11 to 85 days. In this study, as the survival curves obtained did not follow typical first-order destruction kinetics, the primary model chosen was exponential. A second-order polynomial linear regression was then used to study the effects of the various factors on the inhibition of *S*. Typhimurium. Estimated values of the k parameter, which represented the shape of the destruction curves, were well correlated with the predicted ones (R^2 = 0.94). Generally, the higher the temperature with a low pH, the greater the inactivation. With a citric acid concentration of 0.09% (wt/vol), no glucose effect could be seen. In contrast, a lower acid concentration, 0.06% (wt/vol), enabled the higher level of destruction to be reached with a 3.5% (wt/vol) glucose concentration. This study documented that reduced calorie mayonnaise containing citric acid can represent a nonnegligible consumer safety risk and indicated that a well-fitted model is of interest to correctly predict this risk.

147. Messenger, A. M., A. N. Barnes, et al. (2014). "Reverse zoonotic disease transmission (zooanthroponosis): a systematic review of seldom-documented human biological threats to animals." PLoS One **9**(2): e89055. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3938448/pdf/pone.0089055.pdf
BACKGROUND: Research regarding zoonotic diseases often focuses on infectious diseases animals have given to humans. However, an increasing number of reports indicate that humans are transmitting pathogens to animals. Recent examples include methicillin-resistant *Staphylococcus aureus*, influenza A virus, *Cryptosporidium parvum*, and *Ascaris lumbricoides*. The aim of this review was to provide an overview of published literature regarding reverse zoonoses and highlight the need for future work in this area.
METHODS: An initial broad literature review yielded 4763 titles, of which 4704 were excluded as not meeting inclusion criteria. After careful screening, 56 articles (from 56 countries over three decades) with documented human-to-animal disease transmission were included in this report.
FINDINGS: In these publications, 21 (38%) pathogens studied were bacterial, 16 (29%) were viral, 12 (21%) were parasitic, and 7 (13%) were fungal, other, or involved multiple pathogens. Effected animals included wildlife (n = 28, 50%), livestock (n = 24, 43%), companion animals (n = 13, 23%), and various other animals or animals not explicitly mentioned (n = 2, 4%). Published reports of reverse zoonoses transmission occurred in every continent except Antarctica therefore indicating a worldwide disease threat.
INTERPRETATION: As we see a global increase in industrial animal production, the rapid movement of humans and animals, and the habitats of humans and wild animals intertwining with great complexity, the future promises more opportunities for humans to cause reverse zoonoses. Scientific research must be conducted in this area to provide a richer understanding of emerging and reemerging disease threats. As a result, multidisciplinary approaches such as One Health will be needed to mitigate these problems.

148. Messens, W., K. Grijspeerdt, et al. (2005). "Eggshell penetration by *Salmonella*: a review." World's Poult Sci J **61**(01): 71-86.

http://dx.doi.org/10.1079/WPS200443
Intact eggs can become contaminated with *Salmonella* as a result of infections of the reproductive tissues of the laying hens but also by penetration through the shell. In this paper, the penetration of *Salmonella* through the shell of hen eggs is reviewed. A description is given of the advantages and disadvantages of the various methods used to study bacterial penetration through the shell and membranes. The possibility of *Salmonella* contamination of the shell after lay is included because shell contamination is the first requisite for penetration. Various factors affect the probability of bacterial penetration. Both the intrinsic and extrinsic factors are highlighted. For the extrinsic factors, the influence of bacterial strain and number of organisms, temperature, moisture and immersion and storage conditions on the probability of *Salmonella* penetration through the shell is described. With regard to intrinsic factors, the presence of cuticle, shell characteristics (shell quality, porosity, shell defects) and membrane properties are summarized.

149. Messens, W., K. Grijspeerdt, et al. (2006). "Eggshell penetration of hen's eggs by *Salmonella enterica* serovar Enteritidis upon various storage conditions."
Br Poult Sci **47**(5): 554-560.
http://www.ncbi.nlm.nih.gov/pubmed/17050098
1. The survival and penetration of *Salmonella enterica* serovar Enteritidis (SE) inoculated on the eggshell was examined upon storage for up to 20 d at real-life conditions (15 to 25 degrees C and 45 to 75% relative humidity (RH)).
2. Penetration was assessed by emptying the egg contents and filling the eggs with a selective medium that allowed visualising *Salmonella* growth on the inside of the shell and membrane complex.
3. The study of survival on the eggshells was based on viable counts and showed that numbers of surviving organisms decreased over time. Survival was inversely related to storage temperature and RH. Although the average counts decreased over time, a limited proportion of shells carried high numbers of SE at all storage conditions.
4. Penetration spots were observed earlier using an increased storage temperature due to increased growth rates of SE on the agar. After 20 d of storage a similar percentage (c. 44.7%) of eggshells became penetrated, irrespective of the storage conditions tested in this study.
5. The higher the *Salmonella* shell contamination at the end of storage, the higher the probability that the eggshell was penetrated. *Salmonella* shell counts exceeding 4 log cfu yielded more than a 90% probability of eggshell penetration occurring.

150. Methner, U., A. Berndt, et al. (2001). "Combination of competitive exclusion and immunization with an attenuated live *Salmonella* vaccine strain in chickens."
Avian Dis **45**(3): 631-638.
http://www.ncbi.nlm.nih.gov/pubmed/11569736
To use the advantages of both the competitive exclusion (CE) technique and immunization with a live *Salmonella* vaccine, the combination of these methods was studied. Specific-pathogen-free chickens were pretreated by combined or single administration of a CE culture and a commercial live *Salmonella* Typhimurium vaccine on days 1 and 2 of life and challenged with *Salmonella* Typhimurium on day 3 to study the exclusion effect by both the CE preparation and the *Salmonella* vaccine. The exclusion effect by the CE culture combined with the immunologic effect by the live vaccine was studied after challenge of the birds on day 43 of age. The number of challenge organisms in ceca was used to evaluate the efficacy of the pretreatment. The protective exclusion effect of the CE culture was substantial in very young chicks and still detectable in 6-wk-old birds. The attenuated *Salmonella* Typhimurium vaccine produced only an initially occurring exclusion effect. Because the exclusion effect of the CE culture was considerably stronger than the exclusion effect of the attenuated *Salmonella* Typhimurium vaccine, the combination of both did not result in an additive protective effect. In order to exploit the exclusion potential between *Salmonella* strains and to attain an additive exclusion effect by a CE culture and a vaccine strain, live *Salmonella* vaccines are needed that are sufficiently attenuated without affecting genes essential for colonization exclusion of other *Salmonella* organisms. In 6-wk-old birds, the exclusion effect by the CE culture combined with the immunologic effect by the live *Salmonella* vaccine resulted in a degree of protection considerably beyond that generated by the exclusive use of the two methods. The administration of the live *Salmonella* vaccine strain prior to or

simultaneously with the CE culture revealed the best protective effect because such combinations ensure an adequate persistence of the vaccine strain as prerequisite for the expression of an exclusion effect in very young chicks and the development of a strong immune response affording protection in older birds.

151. Mitchell, T. R. and T. Ridgwell (1971). "The frequency of salmonellae in wild ducks." J Med Microbiol **4**(3): 359-361. http://jmm.sgmjournals.org/content/4/3/359.full.pdf
The faecal excretion rate of salmonellae in migratory wild ducks is higher than previous studies would indicate. Examination of 477 duck droppings during the winters of 1968/69 and 1969/70 gave isolation rates of just over 4 per cent. The commonest serotype was *S.* typhimurium; seven different phage-types were found, 2a being the most frequent.

152. Miwa, N., H. Konuma, et al. (2013). "Survival of *Salmonella* Enteritidis on four types of stainless steel surface under a dry condition and recovery by swabbing." Shokuhin Eiseigaku Zasshi **54**(3): 219-223. https://www.jstage.jst.go.jp/article/shokueishi/54/3/54_219/_article
The survival and recovery of *Salmonella* Enteritidis inoculated on stainless steel surfaces with different metal contents and surface finishes were examined. Two *S.* Enteritidis strains possessing different levels of biofilm productivity were inoculated with tryptone soya broth (TSB) and egg yolk emulsion (EY) on the surface of stainless steel squares (1 cm x 1 cm) and stored at 22 under a dry condition. After storage, cells were recovered from the stainless steel surfaces by swabbing with a cotton swab. The numbers of cells recovered by swabbing and the cells remaining on the stainless steel squares were counted. The survival ratio of the strain possessing high biofilm productivity was greater than that of the strain possessing low biofilm productivity. The survival ratio of *S.* Enteritidis suspended in TSB was often higher than that in EY. There were no significant differences in the survival and recovery ratios of *S.* Enteritidis based on stainless steel composition or surface finish. From all except one sample, more than 98% of viable cells of *S.* Enteritidis were recovered by swabbing with a cotton swab.

153. Mohsenin, N. N. (1980). Thermal properties of foods and agricultural materials. NY, New York, Gordon and Breach, Science Publisher, Inc.

154. Moretro, T., L. K. Vestby, et al. (2009). "Evaluation of efficacy of disinfectants against *Salmonella* from the feed industry." J Appl Microbiol **106**(3): 1005-1012. http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2672.2008.04067.x/full
AIMS: To evaluate disinfectants against *Salmonella* under conditions relevant for the feed industry.
MATERIALS AND RESULTS: A survey on the use of disinfectants in feed industry showed that a range of different types was used. Nine disinfectants, reflecting the most commonly used active ingredients, were tested for bactericidal activity on *Salmonella* isolated from the feed industry. All disinfectants were efficient against *Salmonella* in suspension. The bactericidal effect varied considerably between different types of active compounds on bacteria dried on surfaces or grown as biofilm. Tenside-based disinfectants and hypochlorite were found to have low bactericidal activity and the efficiency was significantly reduced when the ratio of amount disinfectant per cell decreased. It was shown that concentrations of 70-80% ethanol were effective against *Salmonella*. Among the disinfectants tested a product containing 70% ethanol was most efficient followed by Virkon S.
CONCLUSIONS: Many disinfectants had low bactericidal activity against *Salmonella* at surfaces while Virkon S and a product containing 70% ethanol were most effective. Another advantage of ethanol-based disinfectants is evaporation of ethanol, resulting in low residual water after use.
SIGNIFICANCE AND IMPACT OF THE STUDY: Use of the disinfectants found to be efficient against surface associated *Salmonella*, may assist the industry in combating *Salmonella*.

155. Morrow, C. (2001). "An integrated approach to *Salmonella* control." International hatchery Practice **16**(4): 11-15

156. Mossel, D. A. A., J. E. L. Corry, et al. (1995). Evaluation of the efficacy of measures to ensure wholesomeness and quality of food by assessing compliance with reference values (`standards'). Essentials of the microbiology of foods : a textbook for advanced studies Chichester, England: John Wiley.

157. Musgrove, M. T., D. R. Jones, et al. (2004). "Identification of *Enterobacteriaceae* from washed and unwashed commercial shell eggs."
J Food Prot **67**(11): 2613-2616.
http://www.ncbi.nlm.nih.gov/pubmed/15553650
To evaluate the effect of processing on the safety and quality of retail shell eggs, a storage study was conducted with unwashed and commercially washed eggs. This work demonstrated that commercial processing decreased microbial contamination of eggshells. To know which species persisted during storage on washed or unwashed eggs, Enterobacteriaceae isolates were selected and identified biochemically. For each of three replications, shell eggs were purchased from a commercial processing plant, transported back to the laboratory, and stored at 4 degrees C. Once a week for 6 weeks, 12 eggs for each treatment (washed and unwashed control) were rinsed in sterile phosphate-buffered saline. A 1-ml aliquot of each sample was plated onto violet red bile glucose agar with overlay and incubated at 37 degrees C for 24 h. Following incubation, plates were observed for colonies characteristic of the family *Enterobacteriaceae*. A maximum of 10 isolates per positive sample were streaked for isolation before being identified to the genus or species level using commercially available biochemical strips. Although most of the isolates from the unwashed control eggs belonged to the genera *Escherichia* or *Enterobacter*, many other genera and species were identified. These included *Citrobacter*, *Klebsiella*, *Kluyvera*, *Pantoea*, *Providencia*, *Rahnella*, *Salmonella*, *Serratia*, and *Yersinia*. Non-Enterobacteriaceae also recovered from the unwashed egg samples included *Xanthomonas* and *Flavimonas*. Very few washed egg samples were contaminated with any of these bacteria. These data provide useful information on the effectiveness of processing in removing microorganisms from commercial shell eggs.

158. Musgrove, M. T., D. R. Jones, et al. (2004). "Survey of shell egg processing plant sanitation programs: effects on non-egg-contact surfaces."
J Food Prot **67**(12): 2801-2804.
http://www.ncbi.nlm.nih.gov/pubmed/15633690
To successfully implement a hazard analysis critical control point plan, prerequisite programs are essential. Sanitation standard operating procedures are an important part of such a plan and can reduce contamination levels so that food safety and quality are not adversely affected. Noncontact surfaces in the shell egg processing plants can serve as a reservoir of cross-contamination. The objective of this study was to assess the efficacy of sanitation programs used in a variety of shell egg processing facilities (in-line, off-line, and mixed operations). Fourteen different noncontact surfaces were sampled in nine commercial facilities across the southeastern United States. Non-egg-contact surfaces were defined as those where the shell egg does not come into direct contact with the surface or with the fluid from that surface. Gauze pads soaked in sterile phosphate-buffered saline were used for sampling at the end of a processing day (POST) and again the next morning prior to operations (PRE). Aerobic plate counts (APCs) and numbers of *Enterobacteriaceae* were determined. No significant differences ($P > 0.05$) were found between POST and PRE counts for either population recovered from the 14 sampling sites. Only samples from the floor under the farm belts, nest-run loader, washers, and packer heads were reduced by 1 log CFU/ml of rinsate for APCs or *Enterobacteriaceae* counts. APCs of more than 10(4) CFU/ml of rinsate were recovered from many samples. Highest APCs were found on the floor under the farm belt and on shelves of the nest-run carts. High APCs were found on the wheel surface for off-line carts and on the loading dock floor. Highest Enterobacteriaceae counts were found in samples from the floor, drain, and nest-run egg cart shelves. A lack of significant difference between POST and PRE counts indicates that current sanitation programs could be improved. These data suggest that traffic patterns for the movement of eggs and materials through the plant should be reevaluated so that cross-contamination is reduced.

159. Musgrove, M. T., D. R. Jones, et al. (2005). "Impact of commercial processing on the microbiology of shell eggs."

J Food Prot **68**(11): 2367-2375.
http://www.ncbi.nlm.nih.gov/pubmed/16300075
Shell egg microbiology has been studied extensively, but little information is available on how modern U.S. processing conditions impact microbial populations. As regulations are being drafted for the industry, such information can be important for determining processing steps critical to product safety. Five different shell egg surface microbial populations (aerobic bacteria, yeasts and molds, *Enterobacteriaceae*, *Escherichia coli*, and *Salmonella*) were monitored at 12 points along the processing line (accumulator, prewash rinse, washer 1, washer 2, sanitizer, dryer, oiler, scales, two packer head lanes, rewash entrance, and rewash exit). Three commercial facilities were each visited three times, a total of 990 eggs were sampled, and 5,220 microbiological samples were subsequently analyzed. Although variations existed in concentrations of microorganisms recovered from each plant, the patterns of fluctuation for each population were similar at each plant. On average, aerobes, yeasts and molds, *Enterobacteriaceae*, and *E. coli* prevalence were reduced by 30, 20, 50, and 30%, respectively, by the end of processing. The microbial concentrations (log CFU per milliliter) in the egg rinse collected from packer head lanes were decreased by 3.3, 1.3, 1.3, and 0.5, respectively, when compared with those of rinses collected from eggs at the accumulator. *Salmonella* was recovered from 0 to 48% of pooled samples in the three repetitions. Higher concentrations of *Salmonella* were recovered from preprocessed than from in-process or ready-to-pack eggs. These data indicate that current commercial practices decrease microbial contamination of egg shell surfaces.

160. Musgrove, M. T., D. R. Jones, et al. (2009). "*Enterobacteriaceae* and related organisms isolated from nest run cart shelves in commercial shell egg processing facilities."
Poult Sci **88**(10): 2113-2117.
http://ps.oxfordjournals.org/content/88/10/2113.long
Enterobacteriaceae, including *Salmonella*, may be recovered from foods and processing facilities. High levels of *Enterobacteriaceae* in the processing plant environment can be an indication of inadequate sanitation. This experiment was designed to determine if nest run egg carts serve as reservoirs for *Enterobacteriaceae*. Eggs that are produced by hens not housed in buildings connected to the processing plant are referred to as nest run. Many of these eggs are transported to the plant on carts to be processed. Two plants in the southeastern United States were sampled. On each of 3 visits, 5 shelves on each of 5 carts were sampled (n=25/visit). A 12x12 cm area on each shelf was swabbed with a sterile gauze pad moistened with PBS and transported on ice back to the laboratory. *Enterobacteriaceae* were enumerated using violet red bile glucose agar incubated at 37 degrees C for 24 h. There was 100% prevalence for *Enterobacteriaceae* at plant A with an average 3.8 log10 cfu/mL swab diluent. Plant B had 90% prevalence for *Enterobacteriaceae* with an average 3.2 log10 cfu/mL swab diluent. Two randomly selected isolates from each positive sample were recultured 3 times to increase the likelihood of clonality and were then identified biochemically. Of the 124 isolates analyzed, genera identified were *Citrobacter* spp., *Escherichia* spp., *Enterobacter* spp., *Klebsiella* spp., *Hafnia* spp., *Kluyvera* spp., *Leclercia* spp., and *Salmonella* spp. *Pseudomonas* spp. was the only non-Enterobacteriaceae identified by our methods. This work demonstrates that nest run egg carts serve as reservoirs for *Enterobacteriaceae* in the shell egg processing environment.

161. Musgrove, M. T., J. K. Northcutt, et al. (2008). "*Enterobacteriaceae* and related organisms isolated from shell eggs collected during commercial processing."
Poult Sci **87**(6): 1211-1218.
http://ps.oxfordjournals.org/content/87/6/1211.long
In the United States, commercial shell eggs are washed and graded before retail. Since passage of the Egg Products Inspection Act in 1971, processing guidelines have been set to ensure that external and internal characteristics are maintained. However, less is known about how commercial processing affects the safety of shell eggs. To identify enteric bacteria entering plants and persisting throughout processing, eggs were collected from 3 US commercial shell egg-processing plants on 3 separate visits. On each plant visit, 12 eggs were collected from each of 12 sites along the processing line: accumulator, prewash rinse, first washer, second washer, sanitizer rinse, dryer, oiler, check detection/scales, 2 egg grader/packer head lanes, rewash belt entrance, and rewash belt exit. Each egg was sampled by a rinse technique, and the rinsate was plated onto violet red bile glucose agar with overlay

for the detection and enumeration of *Enterobacteriaceae*. From each plate, up to 5 colonies were randomly selected and isolated for identification to genus or species by using biochemical tests. Several genera and species were detected at each of the 3 plants. Sites from which the greatest numbers of isolates were identified were those collected from eggs during preprocessing (accumulator, prewash rinse) or from eggs judged as dirty (rewash belt entrance or exit). Sites yielding the smallest number of isolates were those during or at the end of processing. *Escherichia coli* and *Enterobacter* spp. were isolated from each of the 9 plant visits. Other genera isolated from at least 1 of the 3 plants included *Cedecea*, *Citrobacter*, *Erwinia*, *Hafnia*, *Klebsiella*, *Kluyvera*, *Leclercia*, *Morganella*, *Proteus*, *Providencia*, *Rahnella*, *Salmonella*, and *Serratia*. Non-Enterobacteriaceae isolated and identified included *Aeromonas*, *Chryseomonas*, *Listonella*, *Pseudomonas*, *Sphingobacterium*, *Vibrio*, and *Xanthomonas*. All of the genera and species were recovered less frequently from fully processed eggs than from unwashed eggs, indicating that shell eggs are less contaminated with bacteria as a result of commercial washing procedures.

162. Nakamura, M., N. Nagamine, et al. (1994). "Horizontal transmission of *Salmonella* Enteritidis and effect of stress on shedding in laying hens."
Avian Dis **38**(2): 282-288.
http://www.ncbi.nlm.nih.gov/pubmed/7980276
Horizontal transmission of *Salmonella* Enteritidis in laying hens and the short-term effect of stress on shedding were examined in 32 seven-month-old laying hens. Half were inoculated with 10(5) colony-forming units of *S*. Enteritidis phage type 4, and the remaining half were left uninoculated to study horizontal transmission. Isolation of *S*. Enteritidis from cecal droppings of all hens was attempted every morning. Uninoculated hens rapidly became infected through contaminated drinking water. Introduction of young chickens to the same rearing room and withdrawal of water and feed for 2 days coincided with a rapid increase in the shedding rate of *S.* Enteritidis for a short period of time. The results showed that a short-term increase in the shedding rate of *S*. Enteritidis is associated with short-term exposure to environmental stress.

163. Nakamura, M., N. Nagamine, et al. (1994). "Evaluation of the efficacy of a bacterin against *Salmonella* Enteritidis infection and the effect of stress after vaccination."
Avian Dis **38**(4): 717-724.
http://www.ncbi.nlm.nih.gov/pubmed/7702503
An oil-emulsion vaccine was prepared from phage type 4 *Salmonella* Enteritidis to assess the potential protective efficacy of *S*. Enteritidis vaccine and the effect of stress after vaccination. Hens were vaccinated at 14 and 18 weeks of age and challenged orally at 24 weeks of age with 10(6) or 10(3) cells of a homologous *S.* Enteritidis strain. Pullets were vaccinated at 8 and 12 weeks of age and challenged with 10(9) cells. The O antibody titers of vaccinated hens rose quickly and were unchanged after challenge. *S.* Enteritidis was isolated from cecal droppings of significantly fewer vaccinated hens than unvaccinated control hens for 6-21 days and 2-15 days after challenge with 10(6) and 10(3) cells, respectively. *S.* Enteritidis was also isolated from livers and spleens of significantly fewer vaccinated pullets for 1 week. Vaccinated and control hens were equally affected by exposure to environmental stress, the result being that the shedding rate of *S*. enteritidis in cecal droppings increased equally in both groups.

164. Namata, H., E. Meroc, et al. (2008). "*Salmonella* in Belgian laying hens: an identification of risk factors."
Prev Vet Med **83**(3-4): 323-336.
http://www.ncbi.nlm.nih.gov/pubmed/17961763
Since the 1980s, the prevalence of *Salmonella* in Belgian poultry layers and broilers has greatly fluctuated with a rise observed in 2003 and a significant decrease in 2005. In order to alleviate the risk at egg consumer level, it is crucial to understand the factors which influence the contamination and the spread of *Salmonella* in laying hens. To study such determinants we explored the Belgian data from the 2005 baseline study on the prevalence of *Salmonella* in laying flocks of *Gallus gallus* in the European Union. The response variables corresponded to presence or absence of *Salmonella* from dust and faecal samples taken from the environment of a Belgian layer flock. The explanatory variables included: region of Belgium, sampling time (month the flock was sampled), production type (cage or barn and free range), *Salmonella* vaccination status, flock age and flock size. Analyses of these data were

performed using a bivariate logistic regression model assuming independence between the two responses and bivariate generalized estimating equations model, which incorporates the correlation between the two responses on the same flock. The main risk factor that was identified was rearing flocks in cages compared to barns and free-range systems. The results also showed a significant higher risk for *Salmonella* for a 1 week increase in flocks' age as well as with a unit increase in the size of the flock.

165. Nastasi, A., C. Mammina, et al. (1993). "Epidemiology of *Salmonella* Typhimurium: ribosomal DNA analysis of strains from human and animal sources."
Epidemiol Infect **110**(3): 553-565.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2272276/pdf/epidinfect00039-0125.pdf
Salmonella Typhimurium is the most frequently identified serovar of *Salmonella* in Italy. This serovar is characterized by the widespread dissemination among human and non-human sources of phenotypically and genetically well-differentiated clones. In this study 457 strains of *S.* Typhimurium isolated in Italy in the years 1982-91 from human and animal sources were submitted to characterization by the rDNA fingerprinting technique. Application of this typing method, after digestion of chromosomal DNA with HincII endonuclease, confirmed the greatest genetic differentiation of clones of *S.* Typhimurium, allowing reliable identification of 45 rDNA patterns linked into 9 major clusters. rDNA pattern clusters or ribotypes specific to man were not recognized, whereas some rDNA patterns were characteristically related to ducks, pigeons and pet birds. The ribotyping results for isolates from animal hosts suggest that pig and cattle are the main source of human infection.

166. Northcutt, J. K., D. R. Jones, et al. (2004). "Airborne microorganisms in commercial shell egg processing facilities."
Int J Poult Sci **3**(3): 195-200.
http://www.pjbs.org/ijps/fin173.pdf
Total aerobic bacteria, molds/yeasts, coliforms and pseudomonads in the air in three shell egg processing operations (in-line, off-line and mixed operations) were determined using MicroBio MB2 Air Samplers. Sites were sampled from each facility on three different days (replication) during the same week. Four air samples (1000 L each) were drawn from each sampling site on a given day. Sampling sites, included areas in or near the following on-site locations: hen house (in-line and mixed operations), farm transition room (in-line and mixed operations), egg washers, egg dryer, packer heads, post-processing cooler, nest-run cooler (off-line and mixed operations), loading dock and dry storage. Type of operation (in-line, off-line or mixed), sampling site and the interaction between operation and site had a significant effect on the number of total aerobic bacteria, molds/yeasts, coliforms and pseudomonads recovered ($P < 0.05$). Highest counts for total aerobic bacteria (5.9 log10 cfu/ml air), molds/yeasts (4.0 log10 cfu/ml air) and coliforms (2.5 log10 cfu/ml air) were found in the hen house. Highest counts for pseudomonads were found in the hen house (3.2 log10 cfu/ml air) and behind the egg washer (3.5 log10 cfu/ml air). Lowest counts for total aerobic bacteria (2.5 log10 cfu/ml air) and molds/yeast (2.7 log10 cfu/ml air) were found in the post-processing cooler. Few samples in the post-processing coolers, nest-run coolers, loading docks and dry storage areas tested positive for coliforms (0/36, 2/24, 1/36 and 0/36, respectively) and pseudomonads (1/36, 2/24, 5/36 and 6/36, respectively). Data gathered during this study has been useful in identifying the sources and levels of airborne contaminates in commercial shell egg processing facilities.

167. Norton, S., E. Huhtinen, et al. (2012). "A large point-source outbreak of *Salmonella* Typhimurium linked to chicken, pork and salad rolls from a Vietnamese bakery in Sydney."
Western Pac Surveill Response J **3**(2): 16-23.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3729077/pdf/WPSAR.2012.3.2-016.pdf
INTRODUCTION: In January 2011, Sydney South West Public Health Unit was notified of a large number of people presenting with gastroenteritis over two days at a local hospital emergency department (ED).
METHODS: Case-finding was conducted through hospital EDs and general practitioners, which resulted in the notification of 154 possible cases, from which 83 outbreak cases were identified. Fifty-eight cases were interviewed about demographics, symptom profile and food histories. Stool samples were collected and submitted for analysis. An inspection was conducted at a Vietnamese bakery and food samples were collected and submitted for

analysis. Further case ascertainment occurred to ensure control measures were successful. RESULTS: Of the 58 interviewed cases, the symptom profile included diarrhoea (100%), fever (79.3%) and vomiting (89.7%). *Salmonella* Typhimurium multiple-locus-variable number tandem repeats analysis (MLVA) type 3-10-8-9-523 was identified in 95.9% (47/49) of stool samples. Cases reported consuming chicken, pork or salad rolls from a single Vietnamese bakery. Environmental swabs detected widespread contamination with Salmonella at the premises.
DISCUSSION: This was a large point-source outbreak associated with the consumption of Vietnamese-style pork, chicken and salad rolls. These foods have been responsible for significant outbreaks in the past. The typical ingredients of raw egg butter or mayonnaise and pate are often implicated, as are the food-handling practices in food outlets. This indicates the need for education in better food-handling practices, including the benefits of using safer products. Ongoing surveillance will monitor the success of new food regulations introduced in New South Wales during 2011 for improving food-handling practices and reducing foodborne illness.

168. Oakley, B. B., R. J. Buhr, et al. (2014). "Successional changes in the chicken cecal microbiome during 42 days of growth are independent of organic acid feed additives." BMC Vet Res **10**: 282.
http://www.biomedcentral.com/1746-6148/10/282
BACKGROUND: Poultry remains a major source of foodborne bacterial infections. A variety of additives with presumed anti-microbial and/or growth-promoting effects are commonly added to poultry feed during commercial grow-out, yet the effects of these additives on the gastrointestinal microbial community (the GI microbiome) as the bird matures remain largely unknown. Here we compared temporal changes in the cecal microbiome to the effects of formic acid, propionic acid, and medium-chain fatty acids (MCFA) added to feed and/or drinking water.
RESULTS: Cecal bacterial communities at day of hatch (n = 5 birds), 7d (n = 32), 21d (n = 27), and 42d (n = 36) post-hatch were surveyed using direct 454 sequencing of 16S rRNA gene amplicons from each bird in combination with cultivation-based recovery of a *Salmonella* Typhimurium marker strain and quantitative-PCR targeting *Clostridium perfringens*. Treatment effects on specific pathogens were generally non-significant. *S*. Typhimurium introduced by oral gavage at day of hatch was recovered by cultivation from nearly all birds sampled across treatments at 7d and 21d, but by 42d, *S*. Typhimurium was only recovered from ca. 25% of birds, regardless of treatment. Sequencing data also revealed non-significant treatment effects on genera containing known pathogens and on the cecal microbiome as a whole. In contrast, temporal changes in the cecal microbiome were dramatic, highly significant, and consistent across treatments. At 7d, the cecal community was dominated by three genera (*Flavonifractor*, *Pseudoflavonifractor*, and a *Lachnospiracea* sequence type) that accounted for more than half of sequences. By 21d post-hatch, a single genus (Faecalibacterium) accounted for 23-55% of sequences, and the number of *Clostridium* 16S rRNA gene copies detected by quantitative-PCR reached a maximum.
CONCLUSIONS: Over the 42 d experiment, the cecal bacterial community changed significantly as measured by a variety of ecological metrics and increases in the complexity of co-occurrence networks. Management of poultry to improve animal health, nutrition, or food safety may need to consider the interactive effects of any treatments with the dramatic temporal shifts in the taxonomic composition of the cecal microbiome as described here.

169. OIE, W. O. f. A. H. (2014). Biosecurity procedures in the poultry industry. Terrestrial Animal Health Code.
Infectious agents of poultry are a threat to poultry health and, at times, human health and have significant social and economic implications. In poultry production, specially under intensive conditions, prevention is the most viable and economically feasible approach to the control of infectious agents. Biosecurity procedures should be implemented with the objective of preventing the introduction and dissemination of infectious agents in the poultry production chain. Biosecurity will be enhanced with the adoption and implementation of the principles of Good Agricultural Practices and the Hazard Analysis Critical Control Point (HACCP) system.

170. OIE, W. O. f. A. H. (2014). Prevention, detection and control of *Salmonella* in poultry. Terrestrial Animal Health Code.

171. Okamura, M., S. Kikuchi, et al. (2008). "Effect of fixed or changing temperatures during prolonged storage on the growth of *Salmonella enterica* serovar Enteritidis inoculated artificially into shell eggs."
Epidemiol Infect **136**(9): 1210-1216.
http://www.ncbi.nlm.nih.gov/pubmed/17988424
The fate of *Salmonella enterica* serovar Enteritidis (SE) in whole, unbroken eggs was monitored during storage at fixed or changing temperatures after inoculation with 20-47 c.f.u. of SE. Eggs stored at 10 degrees C and 20 degrees C showed little or no bacterial growth over 6 weeks, while egg storage at 30 degrees C increased the percentage of the eggs that contained >10(6) c.f.u. after 3 weeks. Egg storage at 20 degrees C for 5 days followed by 10 degrees C caused only a few eggs with >10(6) c.f.u. after 2 weeks, whereas storage at 22-30 degrees C or 27-35 degrees C for 5 days followed by 25 degrees C induced a rapid increase of eggs that contained >10(6) c.f.u. after 1 or 2 weeks, respectively. Therefore, egg storage at 10 degrees C and 20 degrees C can control SE growth, although the temperature during egg storage and transportation from farm to table should also be taken into consideration.

172. OzFoodNet (2012) Guidelines for the investigation and management of food handlers during non-typhoidal *Salmonella* outbreaks.
Communicable Diseases Surveillance
The approach to testing food handlers during Salmonella outbreaks and the response to food handlers who have Salmonella identified in stool has varied within and between jurisdictions in Australia. A consistent evidence-based approach is desirable. This paper, prepared by an OzFoodNet working group, reviews the available evidence and makes recommendations to guide those investigating and managing non-typhoidal salmonellosis involving food handlers. Infections with Salmonella Typhi and Paratyphi are not considered.

173. Park, S., S. Choi, et al. (2015). "Fate of mesophilic aerobic bacteria and *Salmonella enterica* on the surface of eggs as affected by chicken feces, storage temperature, and relative humidity."
Food Microbiol **48**: 200-205.
http://www.ncbi.nlm.nih.gov/pubmed/25791009
We compared the microbiological quality of chicken eggshells obtained from a traditional wholesale market and a modern supermarket. We also determined the survival and growth characteristics of naturally occurring mesophilic aerobic bacteria (MAB) and artificially inoculated *Salmonella enterica* on eggshells under various environmental conditions (presence of chicken feces, temperature [4, 12, or 25 degrees C], and relative humidity [RH; 43 or 85%]). The populations of MAB, coliforms, and molds and yeasts on eggshells purchased from a traditional wholesale market were significantly (P </= 0.05) higher than those from a modern supermarket. In the second study, when we stored uninoculated eggs under various storage conditions, the population of MAB on eggshells (4.7-4.9 log CFU/egg) remained constant for 21 days, regardless of storage conditions. However, when eggshells were inoculated with *S. enterica* and stored under the same conditions, populations of the pathogen decreased significantly (P </= 0.05) under all tested conditions. Survival of *S. enterica* increased significantly (P </= 0.05) in the presence of feces, at low temperatures, and at low RH. These observations will be of value when predicting the behavior of microorganisms on eggshells and selecting storage conditions that reduce the populations of *S. enterica* on eggshells during distribution.

174. Pennycott, T. W., A. Park, et al. (2006). "Isolation of different serovars of *Salmonella enterica* from wild birds in Great Britain between 1995 and 2003."
Vet Rec **158**(24): 817-820.
http://www.ncbi.nlm.nih.gov/pubmed/16782854
Postmortem examinations were carried out on the carcases of 779 wild birds. Salmonellosis was a common cause of death in greenfinches (*Carduelis chloris*), house sparrows (*Passer domesticus*) and chaffinches (*Fringilla coelebs*), and was also responsible for the deaths of other birds such as goldfinches (*Carduelis carduelis*), feral pigeons and different species of gulls. Most cases of salmonellosis in finches occurred between January and March, whereas salmonellosis in house sparrows tended to occur between October and March. *Salmonella* Typhimurium DT40 and DT56 (variant) predominated in finches and sparrows, DT41 and

DT195 were the most common strains isolated from gulls, and DT2 and DT99 were recovered from feral pigeons. These "wild bird" strains of *Salmonella* made up less than 0.5 per cent of the isolates of *Salmonella* recovered from cattle, sheep, pigs, chickens or turkeys in Great Britain over the same period, but they made up nearly 3 per cent of the isolates from more extensively reared avian livestock such as gamebirds, ducks and geese.

175. Poel, A. F. B. v. d. (2001). "The challenge of sanitising feed."
Feed Technology **4**(10): 16-19
Recent years have proved that the concept of feed quality has become of increasing importance in the feed-to-food chain where quality is a key issue in every step. Microbial contamination, being one of the economical threats, has gained more attention in order to control moulds and bacteria in livestock feeds. The following issues can be mentioned as aspects in relation to feed hygienic quality: - Safety of feeds by exclusion of diet ingredients; - Removal of low doses of feed antibiotics; - Control of microbial population in the gut; - Scaref or the presence of salmonellae; and - A better composition of manure leading to less contamination of the animal, its product and the environment

176. Poppe, C., C. L. Duncan, et al. (1998). "*Salmonella* contamination of hatching and table eggs: a comparison."
Can J Vet Res **62**(3): 191-198.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1189475/pdf/cjvetres00015-0033.pdf
This study determined and compared Salmonella contamination rates of pools of surplus, early and culled hatching eggs from layer and broiler breeder flocks, and of pools of early and regular table eggs from layer flocks. Each pool contained 6 eggs. Five methods were used for the isolation of *Salmonella*. Nine of 126 pools of culled layer hatching eggs, 2 of 126 pools of surplus layer hatching eggs, and one of 126 pools of early layer hatching eggs were contaminated with *Salmonella*. All 126 pools of broiler breeder surplus, and early and culled hatching eggs tested negative for *Salmonella*. All 168 pools of regular table eggs tested negative for *Salmonella*, whilst one of 84 pools of early table eggs contained *Salmonella* agona. The pools of culled layer hatching eggs and surplus layer hatching eggs that contained *S.* Typhimurium were derived from the same breeder operation. Similarly, the pools of culled and early layer hatching eggs that contained *S*. Heidelberg were derived from one breeder operation. Pools of culled hatching eggs were more frequently contaminated with *Salmonella* than other hatching or table eggs. Pools containing eggs that were both cracked and dirty were more frequently contaminated with *Salmonella* than all other pools of eggs. The overall *Salmonella* contamination rate of the table eggs was 0.07 to 0.4%. Critical control points (macroscopic classification of the eggs as cracked and dirty) were validated microbiologically.

177. Pouillot, R., K. Hoelzer, et al. (2014). "Assessment of the risk of salmonellosis from internally contaminated shell eggs following initial storage at 18°C (65°F), compared with 7°C (45°F)."
Food Microbiol **43**(0): 16-19.
http://www.sciencedirect.com/science/article/pii/S0740002014000872
In the U.S., chicken-breeder farms that supply hatcheries typically store and transport eggs intended for broiler production at a temperature of 18.3°C (65°F). However, in case of surplus, some of these eggs may be diverted to human consumption. According to the U.S. Food and Drug Administration's 'Egg Safety Final Rule,' shell eggs intended for human consumption are required to be held or transported at or below 7.2°C (45°F) ambient temperature beginning 36 h after time of lay. We adapted a risk assessment model developed by the U.S. Department of Agriculture's Food Safety Inspection Service, to quantify human exposure to *Salmonella* Enteritidis and the risk of human salmonellosis if eggs are held and transported at 18.3°C for up to 5.5 days after time of lay, as has been observed when hatchery eggs are diverted to human consumption, rather than held and transported at 7.2°C within 36h after time of lay. Storage at 18.3°C leads to considerable bacterial growth in internally contaminated eggs. The model predicted that more than 10% of internally contaminated eggs would remain contaminated after in-shell pasteurization resulting in a 5-log10 reduction, and that some bacteria would survive after home-cooking. The model predicted that, alternatively, eggs stored at 7.2°C after lay would have limited bacterial growth prior to pasteurization, and Salmonella would be very unlikely to be present after pasteurization. The predicted risk of salmonellosis from the consumption of eggs held and

transported at 18.3°C and subsequently diverted to human consumption is 25 times higher than the risk when eggs are held and transported at 7.2°C.

178. Protais, J., P. Colin, et al. (1996). "Line differences in resistance to *Salmonella* Enteritidis PT4 infection."
Br Poult Sci **37**(2): 329-339.
http://www.ncbi.nlm.nih.gov/pubmed/8773842
1. Four groups of hens, each of a different line, were inoculated at peak of lay, per os in the crop with 1 ml of a suspension containing 10(9) cfu/ml *Salmonella* Enteritidis PT4 (SE). The kinetics of SE contamination in the environment, egg shell and yolk were studied during the first 28 d post inoculation. On the day of slaughter, intestines, caeca, spleen, liver, ovary, oviduct and content were investigated for SE contamination.
2. The commercial egg-type line L2 was found to be the most susceptible to SE. It laid many SE-positive yolks (13.8%) and internal and faecal organs were frequently infected.
3. Certain lines are found to exhibit a degree of resistance to SE; the cause of which is unknown and might be attributed to major genes.

179. Rabie, A. J., I. M. McLaren, et al. (2015). "Assessment of anti-*Salmonella* activity of boot dip samples."
Avian Pathol **44**(2): 129-134.
http://www.ncbi.nlm.nih.gov/pubmed/25650744
The introduction of pathogens from the external environment into poultry houses via the boots of farm workers and visitors presents a significant risk. The use of boot dips containing disinfectant to help prevent this from happening is common practice, but the effectiveness of these boot dips as a preventive measure can vary. The aim of this study was to assess the anti-*Salmonella* activity of boot dips that are being used on poultry farms. Boot dip samples were collected from commercial laying hen farms in the UK and tested within 24 hours of receipt at the laboratory to assess their anti-*Salmonella* activity. All boot dip samples were tested against a field strain of *Salmonella enterica* serovar Enteritidis using three test models: pure culture, paper disc surface matrix and yeast suspension model. Of the 112 boot dip samples tested 83.6% were effective against *Salmonella* in pure culture, 37.3% in paper disc surface matrix and 44.5% in yeast suspension model. Numerous factors may influence the efficacy of the disinfectants. Disinfectants used in the dips may not always be fully active against surface or organic matter contamination; they may be inaccurately measured or diluted to a concentration other than that specified or recommended; dips may not be changed regularly or may have been exposed to rain and other environmental elements. This study showed that boot dips in use on poultry farms are frequently ineffective.

180. Raghiante, F., T. Rocha, et al. (2010). "Penetration time of *Salmonella* Heidelberg through shells of white and brown commercial eggs."
Revista Brasileira de Ciência Avícola **12**: 273-277.
http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-635X2010000400009&nrm=iso
This study aimed at determining the minimum time required for the penetration of *Salmonella* Heidelberg inside the eggs after contact with contaminated material. Recently-collected brown and white eggs from laying hens between 45-50 weeks of age, reared in a commercial poultry house, were artificially contaminated by contact with wood shavings moistened with liquid inoculum of *Salmonella* Heidelberg in stationarygrowth phase (10^3-10^4 CFU g^{-1}). According to type (white or brown), eggs were distributed into three different groups, with four replicates each: negative control group (no artificial contamination), positive control group (analyzed externally immediately after contamination and internally after the maximum storage period of the test group) and test group. Eggs were stored at controlled environmental temperature varying from 25ºC to 30ºC. In the test group, eggs contents (yolk and albumen) were pooled and analyzed after 1:00, 1:30, 2:00, 2:30, 3:00, 3:30, and 4:00 hours after contamination for the presence of *Salmonella* Heidelberg in 25g of this pool. The experimental unit consisted of five eggs in each test. The analysis protocol included pre-enrichment, selective enrichment, plating on selective agar, and biochemical and serological tests. The results obtained were submitted to logistic regression, which indicated that the presence of *Salmonella* Heidelberg was verified after 2:16 h and 2:44 h of contact with white and brown eggs, respectively.

181. Recio, J. I. A., H. Bailie, et al. (2007). "Report of the task force on zoonoses data collection on the analysis of the baseline study on the prevalence of *Salmonella* in holdings of laying hen flocks of *Gallus gallus*."
EFSA Journal **97**: 1-84
http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/97r.pdf

182. Reynolds, A., C. R. Moffatt, et al. (2010). "An outbreak of gastroenteritis due to *Salmonella* Typhimurium phage type 170 associated with consumption of a dessert containing raw egg."
Commun Dis Intell Q Rep **34**(3): 329-333.
http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3403-pdf-cnt.htm/$FILE/cdi3403k.pdf
Eggs are frequently implicated as a source of foodborne salmonellosis. In February 2009 an investigation was commenced following reports of gastrointestinal illness among diners at a Canberra restaurant. The investigation sought to confirm the existence of an outbreak, identify a source and implement public health measures to prevent more cases. Menus and booking lists were obtained from the restaurant and a case-control study was commenced. A suspected case was defined as a person who ate at the restaurant on 13 or 14 February 2009 and subsequently developed diarrhoea and/or vomiting. Twenty cases and 31 controls were enrolled in the study. Eating a tiramisu dessert containing raw egg had a highly statistically significant association with illness (crude odds ratio 130.50, 95% confidence interval 13.54-1605.28). Among the 20 cases, nine of 12 stool samples were positive for *Salmonella* Typhimurium phage type 170 (STm 170). No microbiological evidence of STm 170 was obtained from the restaurant or during the egg trace-back investigation. This report highlights the risk associated with consumption of foods containing raw or undercooked shell egg.

183. Roberts-Witteveen, A. R., B. A. Campbell, et al. (2009). "Egg-associated *Salmonella* outbreak in an aged care facility, New South Wales, 2008."
Commun Dis Intell Q Rep **33**(1): 49-52.
http://www.ncbi.nlm.nih.gov/pubmed/19618772
Salmonellosis is a bacterial disease that causes acute gastroenteritis, with sudden onset of headache, abdominal pain, diarrhoea, nausea and sometimes vomiting. Infection is often associated with the consumption of foods prepared using raw eggs. During July to August 2008 an outbreak at an aged care facility (ACF) in New South Wales was confirmed as *Salmonella* Typhimurium phage type 44 (Stm 44) in eight of 45 residents. Two additional probable cases also occurred. Cases were located in each unit of the ACF and for 5 cases, onset of diarrhoea was between 45 to 64 hours (median of 46 hours) after consumption of a dessert containing raw eggs. Onset for 5 further cases occurred up to 9 days after this meal. Eggs were supplied to the ACF from a local farm. Stm 44 was detected on an egg in an unopened box at the ACF from this supplier. The raw-egg dessert was epidemiologically implicated as the likely source of the *Salmonella* and delayed onset cases may have resulted from ingestion of a smaller dose of *Salmonella*, or ongoing transmission through cross-contamination of kitchen machinery or surfaces. This outbreak demonstrates that inadequate cooking of eggs continues to pose a risk for *Salmonella* infection in settings with vulnerable populations. The findings of the investigation provide support for the importance of food safety regulations and demand further advocacy for measures to reduce the risks associated with the distribution, storage and preparation of shell eggs.

184. Roberts, J. R. (2004). "Factors affecting egg internal quality and egg shell quality in laying hens."
J Poultry Sci **41**(3): 161-177
https://www.jstage.jst.go.jp/article/jpsa/41/3/41_3_161/_pdf
Egg shell quality and egg internal quality are of major importance to the egg industry worldwide. This review covers the formation of the hen's egg and ways of measuring egg shell quality and egg internal quality. Egg shell quality may be measured as egg size, egg specific gravity, shell colour, shell breaking strength, shell deformation (destructive or non-destructive), shell weight, percentage shell, shell thickness, and shell ultrastructure. New methods emerge from time to time. Egg internal quality is measured as yolk colour, the integrity of the perivitelline membrane, and albumen quality. Factors that affect egg shell quality and egg internal quality are reviewed. The complexity of the process of egg shell formation means that imperfections can arise in a number of places in the oviduct of the hen.

Egg shell quality may be affected by the strain and age of hen ; induced moult ; nutritional factors such as calcium, phosphorus, vitamins, water quality, non-starch polysaccharides, enzymes, contamination of feed ; general stress and heat stress ; disease, production system, or addition of proprietary products to the diets. Egg internal quality may be affected by storage ; hen strain and age ; induced moult, nutrition, and disease. An understanding of the range of factors that affect egg shell quality and egg internal quality is essential for the production of eggs of high quality.

185. Roche, A. J., N. A. Cox, et al. (2009). "Transmission of *Salmonella* to broilers by contaminated larval and adult lesser mealworms, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)."
Poult Sci **88**(1): 44-48.
http://ps.oxfordjournals.org/content/88/1/44.full.pdf
The ability of the lesser mealworm, *Alphitobius diaperinus* (Panzer), commonly known as the darkling beetle, to transmit marker *Salmonella* Typhimurium to day-of-hatch broiler chicks was evaluated, as well as the spread to nonchallenged pen mates. In trial 1, day-of-hatch chicks were orally gavaged with 4 larval or 4 adult beetles that had been exposed to marker *Salmonella*-inoculated feed for 72 h. In addition, chicks were gavaged with the marker *Salmonella* in saline solution. These chicks were then placed into pens to serve as challenged broilers. In trial 2, all pens received 2 challenged chicks that were gavaged with larvae or beetles that had been exposed to marker *Salmonella*-inoculated feed for 24 h and then removed from the inoculated feed for a period of 7 d. At 3 wk of age, cecal samples from the marker *Salmonella*-challenged broilers and from 5 pen mates in trial 1, or 10 pen mates in trial 2, were evaluated for the presence of the marker *Salmonella* in their ceca, and at 6 wk of age, all remaining pen mates were sampled. To monitor the presence of the marker *Salmonella* within pens, stepped-on drag swab litter samples were taken weekly. For the *Salmonella*-saline pens, 29 to 33% of the broilers that had been challenged and 10 to 55% of the pen mates were positive at 3 wk of age, and only 2 to 6% had positive ceca at 6 wk. For the pens challenged with adult beetles, 0 to 57% of the challenged broilers and 20 to 40% of the pen mates had positive ceca at 3 wk, and 4 to 7% were positive at 6 wk. The pens challenged with larvae had the greatest percentage of marker *Salmonella*-positive broilers; 25 to 33% of the challenged broilers and 45 to 58% of pen mates were positive at 3 wk, and 11 to 27% were positive at 6 wk. These results demonstrated that ingestion of larval or adult beetles contaminated with a marker *Salmonella* could be a significant vector for transmission to broilers.

186. Rodriguez-Navarro, A. B., N. Dominguez-Gasca, et al. (2013). "Change in the chicken eggshell cuticle with hen age and egg freshness."
Poult Sci **92**(11): 3026-3035.
http://www.ugr.es/~mortega/documentos/PoultryScience2013.pdf
For a fuller understanding of the functionality of the eggshell cuticle, we conducted a detailed study using a wide array of analytical techniques (scanning and transmission microscopy), energy dispersive x-rays, and attenuated total reflection-Fourier transform infrared spectroscopy to analyze the structure, morphology, and chemical composition of this organic coating. This study shows that the cuticle has a compositional gradation with an outer part richer in proteins and an inner part richer in sulfated polysaccharides and phosphates. It also shown that the cuticle composition, thickness, and degree of coverage are highly dependent on hen age and egg freshness. During the course of the first laying year, the thickness and degree of glycosylation of the cuticle decreases with hen age, and at the end of the laying cycle, the cuticle is significantly depleted in lipids. There are also well-defined compositional changes in the cuticle of freshly laid eggs as time passes and there is a notable increase in the permeability of the eggshell after 24 h due to cuticle drying. We discuss how these changes in the cuticle can affect the food safety of eggs in relation to the risk of trans-shell contamination by bacteria (i.e., Salmonellosis).

187. Rule, A. M., S. L. Evans, et al. (2008). "Food animal transport: a potential source of community exposures to health hazards from industrial farming (CAFOs)."
J Infect Public Health **1**(1): 33-39.
http://www.ncbi.nlm.nih.gov/pubmed/20701843

Use of antimicrobial feed additives in food animal production is associated with selection for drug resistance in bacterial pathogens, which can then be released into the environment through occupational exposures, high volume ventilation of animal houses, and land application of animal wastes. We tested the hypothesis that current methods of transporting food animals from farms to slaughterhouses may result in pathogen releases and potential exposures of persons in vehicles traveling on the same road. Air and surface samples were taken from cars driving behind poultry trucks for 17 miles. Air conditioners and fans were turned off and windows fully opened. Background and blank samples were used for quality control. Samples were analyzed for susceptible and drug-resistant strains. Results indicate an increase in the number of total aerobic bacteria including both susceptible and drug-resistant enterococci isolated from air and surface samples, and suggest that food animal transport in open crates introduces a novel route of exposure to harmful microorganisms and may disseminate these pathogens into the general environment. These findings support the need for further exposure characterization, and attention to improving methods of food animal transport, especially in highly trafficked regions of high density farming such as the Delmarva Peninsula.

188. Russell, S. M. (2012). Controlling *Salmonella* in poultry production and processing. Boca Raton, FL, USA, Taylor & Francis Group.
Salmonella is a major pathogen that can result in foodborne illness. This book explains the origin of *Salmonella* on poultry and offers intervention strategies for controlling *Salmonella* during breeding, hatching, grow-out, transportation and processing. The text examines the implications and proper use of chemicals, how to diagnose and tune a processing plant to eliminate *Salmonella*, and how to verify intervention strategies to ensure that they are working. It also discusses the implications of recycling water on *Salmonella* contamination and offers practical tips for increasing yeild during processing while controlling for *Salmonella* and designing the propoer equipment to eliminate *Salmonella*.

189. Samiullah, K. K. Chousalkar, et al. (2013). "Effects of egg shell quality and washing on *Salmonella* Infantis penetration."
Int J Food Microbiol **165**(2): 77-83.
http://www.ncbi.nlm.nih.gov/pubmed/23727650
The vast majority of eggs in Australia are washed prior to packing to remove dirt and fecal material and to reduce the microbial contamination of the egg shell. The egg contents can be an ideal growth medium for microorganisms which can result in human illness if eggs are stored improperly and eaten raw or undercooked, and it is estimated that egg-related salmonellosis is costing Australia $44 million per year. Egg shell characteristics such as shell thickness, amount of cuticle present, and thickness of individual egg shell layers can affect the ease with which bacteria can penetrate the egg shell and washing could partially or completely remove the cuticle layer. The current study was conducted to investigate the effects of egg washing on cuticle cover and effects of egg shell quality and cuticle cover on *Salmonella* Infantis penetration of the egg shell. A higher incidence of unfavorable ultrastructural variables of the mammillary layer such as late fusion, type B bodies, type A bodies, poor cap quality, alignment, depression, erosion and cubics were recorded in *Salmonella* penetrated areas of egg shells. The influence of egg washing on the ability of *Salmonella* Infantis on the egg shell surface to enter the egg internal contents was also investigated using culture-based agar egg penetration and real-time qPCR based experiments. The results from the current study indicate that washing affected cuticle cover. There were no significant differences in *Salmonella* Infantis penetration of washed or unwashed eggs. Egg shell translucency may have effects on *Salmonella* Infantis penetration of the egg shell. The qPCR assay was more sensitive for detection of Salmonella Infantis from egg shell wash and internal contents than traditional microbiological methods. The agar egg and whole egg inoculation experiments indicated that *Salmonella* Infantis penetrated the egg shells. Egg washing not only can be highly effective at removing *Salmonella* Infantis from the egg shell surface, but also allows subsequent trans-shell and trans-membrane penetration into the egg. Consequently, it is important to prevent recontamination of the egg after washing.

190. Sander, J. E., C. L. Hofacre, et al. (2002). "Investigation of resistance of bacteria from commercial poultry sources to commercial disinfectants."

Avian Dis **46**(4): 997-1000.
http://www.ncbi.nlm.nih.gov/pubmed/12495063
Concern by consumers about food safety has resulted in increased pressure on poultry companies to develop effective sanitation programs. *Salmonella* isolates in hatcheries are often the same species isolated from processing plants. Resistance develops in bacteria after prolonged exposure to disinfectants. The methods available in published literature to detect the efficacy of disinfectants are labor intensive and do not consider how bacteria behave when adhered to a solid surface. We used a recently developed technique, which utilizes the actual surfaces on which the disinfectant is to be applied, to evaluate the degree of resistance to four commercially available disinfectants of 17 bacterial isolates from poultry hatcheries. We found that bacterial isolates within the same genus and species have different sensitivities to the same disinfectant. In addition, disinfectants with similar but not identical chemical formulations have different efficacies against the same bacteria.

191. Santo Domingo, J. W., S. Harmon, et al. (2000). "Survival of *Salmonella* species in river water."
Curr Microbiol **40**(6): 409-417.
http://www.ncbi.nlm.nih.gov/pubmed/10827285
The survival of four *Salmonella* strains in river water microcosms was monitored by culturing techniques, direct counts, whole-cell hybridization, scanning electron microscopy, and resuscitation techniques via the direct viable count method and flow cytometry. Plate counts of bacteria resuspended in filtered and untreated river water decreased several orders of magnitude within the first week of incubation, while they did not decrease as rapidly in autoclaved water. In situ hybridization studies suggested a rapid decrease in ribosomal content, as determined by the drastic decrease in the number of detectable cells after 72 h. In contrast, direct counts remained relatively constant during 45 days in all microcosoms. Although the culturable counts of two bacterial strains in filtered water after 31 days represented approximately 0.001% of the total counts, direct viable counts and resuscitation studies with a dilution series suggested that the number of viable bacteria was at least four orders of magnitude higher. Additionally, notable changes in forward scatter and in nucleic acid content were observed only after 4 h of nutrient amendments by flow cytometry. However, cells from the resuscitation experiments did not grow on solid media unless cell-free supernatant from viable cultures was added during the resuscitation period. The results in this study suggest the presence of a not immediately culturable status in *Salmonella*.

192. Sarna, M., G. Dowse, et al. (2002). "An outbreak of *Salmonella* Typhimurium PTI35 gastroenteritis associated with a minimally cooked dessert containing raw eggs."
Commun Dis Intell Q Rep **26**(1): 32-37.
http://www.ncbi.nlm.nih.gov/pubmed/11950199
In April 2000, we investigated an outbreak of gastroenteritis amongst attendees of a local community dinner in a Perth suburb. Of the 98 people interviewed (response rate 98%), 53 reported gastrointestinal symptoms (attack rate 54%). Faecal cultures from 11 cases, 2 food preparers, 1 waitress and leftover mock ice-cream dessert grew *Salmonella* Typhimurium PT135. Of the 3 food handlers, one was asymptomatic, another gave an unclear history of onset of illness and the waitress claimed illness onset 9 days after the dinner. A cohort study implicated fruit salad (RR 1.64 [95% CI: 1.05-2.58], p=0.017) and/or mock ice-cream dessert (RR 1.78 [95% CI: 0.91-3.52], p=0.045). Eggs used to make the mock ice-cream dessert were supplied directly from the producer who used inappropriate shell cleaning methods. The method of preparation of the dessert encouraged contamination. *Salmonella* species were not isolated in poultry faecal samples collected from the implicated egg farm. The cause of this outbreak was almost certainly the ice-cream dessert with contamination most likely resulting either from the eggs used to make the dessert or one or both of the food preparers, coupled with inadequate cooking of the dessert. Eggs used in preparing food for mass consumption should be sourced from distributors with approved cleaning procedures. Furthermore, pasteurised egg products or egg pulp should be used in the preparation of uncooked or minimally cooked dishes.

193. Sauter, E. A. and C. F. Petersen (1974). "The effect of egg shell quality on penetration by various salmonellae."
Poult Sci **53**(6): 2159-2162.

http://ps.oxfordjournals.org/content/53/6/2159.long
Eggs having specific gravity values of 1.070, 1.080 and 1.090 were challenged by 12 species of Salmonellae. Challenge was by dipping eggs for 3 minutes into solutions containing approximately 1 x 10^4 *Salmonella* organisms/ml. Egg temperature was 23° C. with the solution at 5° C. at the time of challenge after which the eggs were allowed to dry at room temperature, then stored at 30° C. for 24 hours prior to sampling for *Salmonella* penetration. Egg shells were sterilized with iodine, then opened and swab samples were incubated for 24 hours at 37° C. in tetrathionate and/or Selenite F broth, then streaked on MacConkey and SS Agar plates to determine presences of Salmonella organisms. Penetration into eggs of low shell quality (1.070) ranged from 14.3% by *S. oranienburg* to 82.1% by *S. typhimurium* and averaged 47.5% of all eggs challenged. Penetration averaged 21.4% of eggs of intermediate shell quality ranging from 7.5% for *S. anatum* and *S. oranienburg* to 48.7% of eggs for *S. typhimurium.* Average penetration was 10% of eggs of excellent shell quality ranging from 3.8% by *S. anatum* to 21.2% by *S. typhimurium.* Addition of 16 p.p.m. of iron to challenge solution increased penetration with five of six species tested.

194. Scher, K., U. Romling, et al. (2005). "Effect of heat, acidification, and chlorination on *Salmonella enterica* serovar typhimurium cells in a biofilm formed at the air-liquid interface." Appl Environ Microbiol **71**(3): 1163-1168.
http://aem.asm.org/content/71/3/1163.full
Bacterial biofilms have great significance for public health, since biofilm-associated microorganisms exhibit dramatically decreased susceptibility to antimicrobial agents and treatments. To date most attention has focused on biofilms that arise from the colonization of solid-liquid or solid-air interfaces. It is of interest that colonization of the interface between air and liquid, which can be selectively advantageous for aerobic or facultative aerobic bacteria, has been rarely studied, although it may present a major problem in industrial aquatic systems. In this work we investigated the role of a biofilm at the interface between air and liquid (pellicle) in the susceptibility of *Salmonella enterica* serovar Typhimurium to stress conditions. For a control we used a mutant that had lost its ability to synthesize cellulose and thin aggregative fimbriae and thus did not produce the pellicle. Resistance of bacteria from the pellicle to heat, acidification, and chlorination was compared to resistance of planktonic cells from the logarithmic and stationary phases of growth. Pellicle cells were significantly more resistant to chlorination, and thus the surrounding matrix conferred protection against the reactive sodium hypochlorite. However, the stress management of pellicle cells in response to heat and low pH was not enhanced compared to that of stationary-phase cells. A long-period of incubation resulted in endogenous hydrolysis of the pellicle matrix. This phenomenon provides a potential new approach to combat microbial cells in biofilms.

195. Schoeni, J. L., K. A. Glass, et al. (1995). "Growth and penetration of *Salmonella* Enteritidis, *Salmonella* Heidelberg and *Salmonella* Typhimurium in eggs." Int J Food Microbiol **24**(3): 385-396.
http://www.ncbi.nlm.nih.gov/pubmed/7710915
Eggs and egg dishes are important vehicles for *Salmonella* infections. *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Heidelberg, which can be isolated from chicken ovaries and feces, have been implicated in approximately 50% of the foodborne salmonellosis outbreaks in the United States. In this study, the growth of these three organisms, inoculated into yolks and albumen, was compared at 4, 10 and 25 degrees C. Regardless of whether 10(2) cfu/g or 10(4) cfu/g was inoculated into the yolk or albumen, populations of all strains increased 3 logs or more in number in one day when incubated at 25 degrees C. Maximum numbers of *Salmonella* ranged from 10(8) to 10(10) cfu/g. All strains grew at 10 degrees C, but peak numbers were lower and occurred later than those at 25 degrees C. Populations of the three *Salmonella* strains inoculated into eggs stored at 4 degrees C grew sporadically; in some test groups populations declined. The potential for *Salmonella* in contaminated feces to establish in the interior of eggs was examined by monitoring shell penetration. At 25 degrees C, all three *Salmonella* strains penetrated the shell in 3 days, but at 4 degrees C, only *S.* Typhimurium was found in one membrane sample. When hatchery conditions were simulated by incubating eggs at 35 degrees C for 30 min followed by storage at 4 degrees C, penetration was enhanced. Penetration was observed by day 1-3 when eggs were exposed to 10(4) cfu *Salmonella*/g feces. Increasing the inoculum to 10(6) cfu/g feces resulted in 50-75% of the contents of eggs to be contaminated by day 1. All *Salmonella*-positive samples were

detected by enrichment. Results of this study indicate that *S.* Enteritidis, *S*. Typhimurium, or *S*. Heidelberg present in feces can penetrate to the interior of eggs and grow during storage.

196. Scott, E. and S. F. Bloomfield (1990). "Investigations of the effectiveness of detergent washing, drying and chemical disinfection on contamination of cleaning cloths." J Appl Bacteriol **68**(3): 279-283. http://www.ncbi.nlm.nih.gov/pubmed/2341327
Detergent washing, drying and chemical disinfection for decontamination of cleaning cloths was investigated with cloths contaminated by use in the domestic environment. Detergent washing produced only limited reductions in microbial contamination and cloths then stored at room temperature for 24 h showed increases in contamination due to multiplication of residual survivors. For effective and consistent decontamination of cloths, detergent washing followed by drying at 80 degrees C for 2 h was required. Hypochlorite and phenolic disinfectants produced significant reductions in contamination, but chemical disinfection may be unreliable where cloths are heavily contaminated.

197. Scott, E. and S. F. Bloomfield (1990). "The survival and transfer of microbial contamination via cloths, hands and utensils." J Appl Bacteriol **68**(3): 271-278. http://www.ncbi.nlm.nih.gov/pubmed/2111304
Survival and transfer of bacteria from laminated surfaces and cleaning cloths were investigated under laboratory conditions. Drying produced substantial reductions in numbers of recoverable organisms and achieved satisfactory decontamination of clean laminate surfaces. On soiled surfaces and on clean and soiled cloths, Gram-positive and some Gram-negative species survived for up to 4 h, and in some cases up to 24 h. Where contaminated surfaces or cloths came into contact with the fingers, a stainless steel bowl, or a clean laminate surface, organisms were transferred in sufficient numbers to represent a potential hazard if in contact with food.

198. Sexton, M. (2012). "Egg washing – Improving efficacy and safety to optimise profitability." Poultry CRC Final Report **3.2.1**
The aims of this project were to improve the recovery of dirty and black eggs and to reduce microbial contamination on the egg shells, as measured by Total Viable Counts (TVC, used as hygiene indicator), *Enterobacteriaceae* (used as a faecal indicator and include *Escherichia coli* and *Salmonella*) and *Salmonella*. These aims were achieved through a series of laboratory and in-plant trials. In the first of three laboratory trials three commercial chemical detergent and sanitiser combinations were assessed for their ability to clean artificially dirtied eggs from two hen ages. The detergent was applied either at 30 or 40°C and the sanitizer at 2°C higher or 8°C lower than the detergent. The two most effective combinations were Circhlor, a liquid alkaline chlorine based product, at 40°C used with Virogard, a liquid quaternary ammonium compound (200 ppm) based sanitiser, at 42°C, and Asepto LF, a liquid sodium hypochlorite based product, at 40°C used with Prochlor, a liquid sodium hypochlorite (200 ppm) based sanitiser.

199. Seydim, A. and P. Dawson (1999). "Packaging effects on shell egg breakage rates during simulated transportation." Poult Sci **78**(1): 148-151. http://ps.oxfordjournals.org/content/78/1/148.long
Shell eggs were packaged in either expanded polystyrene (EPS) foam or molded paper pulp (MPP) one dozen cartons, then were bulk packaged in either polypropylene crates or corrugated boxes. The packages were then subjected to a well-defined computer-simulated vibration test on an electrohydraulic test machine. The percentage and the location on the egg (side, top, bottom) of breakage was determined in the secondary (corrugated box or polypropylene crate) and primary (EPS or MPP carton) package after 15, 75, and 180 min. For each of three trials, 60 dozen Grade A large eggs were randomly assigned to each primary package and cross-stacked in a secondary container that contained three cartons in a row and a total of five layers. When cartons were packed in 15-dozen corrugated boxes, no significant difference was found in total eggshell damage rates between the MPP carton and the EPS carton. However, when eggs were packed in 15-dozen plastic crates, the MPP cartons caused significantly less eggshell damage than the EPS cartons. The EPS cartons

packed in corrugated boxes had the lowest breakage (4.63%), whereas the EPS foam cartons packed in plastic crates had the highest breakage (12.59%). When the effect of secondary packaging and vibration time were not considered, no significant difference was found between MPP and EPS cartons. In addition, when the effect of primary packaging was not taken into account, the corrugated boxes had significantly lower breakage rates than the plastic crates. Nearly 55% of the breakage occurred in the bottom section of the eggshell as compared to the side and top. When the test periods were compared, the EPS cartons packed in plastic crates had the highest breakage (16.28%) at 180 min.

200. Sha, Q., M. R. Forstner, et al. (2013). "Diversity of *Salmonella* in biofilms and water in a headwater ecosystem."
FEMS Microbiol Ecol **83**(3): 642-649.
http://www.ncbi.nlm.nih.gov/pubmed/23025800
The diversity of *Salmonella* was analysed in biofilm and water samples from the spring and slough arms of Spring Lake, the headwaters of the San Marcos River, Texas, during the drought of 2011, with only one potential run-off event at the beginning of the study. Salmonellae were detected in semiselective enrichment cultures by end-point PCR during the entire sampling period of (11 sampling events during 2 months). From the spring arm site, 73% of the biofilms and 41% of the water samples were positive for salmonellae, while only 9% of the biofilms and 23% of the water samples were positive from the slough arm site. Salmonellae could be isolated from all positive samples, with higher diversity in biofilms compared with water samples and more strains obtained from the spring arm than from the slough arm. Differences between sites were generally caused by less frequently detected isolates, while the majority of isolates that were present in both biofilms and water from both sites was represented by three strains only. Quantification attempts by quantitative PCR directly in samples without prior enrichment did not result in a reliable detection of salmonellae, suggesting that numbers in all samples were below the detection limit.

201. Sha, Q., D. A. Vattem, et al. (2013). "Quantifying *Salmonella* population dynamics in water and biofilms."
Microb Ecol **65**(1): 60-67.
http://www.ncbi.nlm.nih.gov/pubmed/22890729
Members of the bacterial genus *Salmonella* are recognized worldwide as major zoonotic pathogens often found to persist in non-enteric environments including heterogeneous aquatic biofilms. In this study, *Salmonella* isolates that had been detected repeatedly over time in aquatic biofilms at different sites in Spring Lake, San Marcos, Texas, were identified as serovars Give, Thompson, Newport and -:z10:z39. Pathogenicity results from feeding studies with the nematode *Caenorhabditis elegans* as host confirmed that these strains were pathogenic, with *Salmonella* fed *C. elegans* dying faster (mean survival time between 3 and 4 days) than controls, i.e., *Escherichia coli* fed *C. elegans* (mean survival time of 9.5 days). Cells of these isolates inoculated into water at a density of up to 10(6) ml(-1) water declined numerically by 3 orders of magnitude within 2 days, reaching the detection limit of our quantitative polymerase chain reaction (qPCR)-based quantification technique (i.e., 10(3) cells ml(-1)). Similar patterns were obtained for cells in heterogeneous aquatic biofilms developed on tiles and originally free of *Salmonella* that were kept in the inoculated water. Cell numbers increased during the first days to more than 10(7) cells cm(-2), and then declined over time. Ten-fold higher cell numbers of *Salmonella* inoculated into water or into biofilm resulted in similar patterns of population dynamics, though cells in biofilms remained detectable with numbers around 10(4) cells cm(-2) after 4 weeks. Independent of detectability by qPCR, samples of all treatments harbored viable salmonellae that resembled the inoculated isolates after 4 weeks of incubation. These results demonstrate that pathogenic salmonellae were isolated from heterogeneous aquatic biofilms and that they could persist and stay viable in such biofilms in high numbers for some time.

202. Shini, S., A. Shini, et al. (2013). "The potential for probiotics to prevent reproductive tract lesions in free-range laying hens."
Anim Prod Sci **53**(12): 1298-1308.
http://www.publish.csiro.au/view/journals/dsp_journal_fulltext.cfm?nid=72&f=AN12337
A study was undertaken to investigate the ability of two commercial probiotics applied in free-range laying hens (from 18 to 22 weeks of age) in reducing the occurrence of reproductive

tract pathologies, and improving hen health and performance. In all, 630 17-week-old brown layers were transferred to a freshly cleaned free-range laying facility, and randomly divided into three groups, with three replicates of 70 birds each. Both probiotics were administered in the drinking water (Groups 1 and 2) on a daily basis for 4 weeks, while Group 3 was left untreated. At 38 weeks of age, the results demonstrated that treatment with either probiotic significantly reduced the occurrence of reproductive tract pathologies (control vs probiotics, 33% vs 22% and 11%; $P < 0.01$), mortalities (control vs probiotics; 3.8% vs 1.5 and 1.9%; $P < 0.01$), and increased the performance of hens, for another 20 weeks post-treatments (hen day production for control vs probiotics 75% vs 90% and 94%; $P < 0.01$). Birds treated with probiotics maintained their bodyweight and egg weights at standard ranges, while untreated birds did not perform at this level. Although we were unable to show any effect on cloacal bacterial colonisation, the results of the present study provided some initial evidence that reproductive pathologies that often cause drops in egg production and sudden deaths of birds, can be reduced if free range hens are treated with a commercial probiotic before or during the onset of lay. The use of a probiotic benefits the health and performance status of hens, resulting in better hen welfare and significant economic gains to egg producers.

203. Simmons, G. C. (1951). "Salmonellosis in domestic animals and birds in Queensland." Aust Vet J **27**: 296-231 http://onlinelibrary.wiley.com/doi/10.1111/j.1751-0813.1951.tb04988.x/abstract

204. Singh, A., N. R. Korasapati, et al. (2011). "Dynamic Predictive Model for the Growth of *Salmonella* spp. in Liquid Whole Egg." J Food Sci **76**(3): M225-M232. http://dx.doi.org/10.1111/j.1750-3841.2011.02074.x
A dynamic model for the growth of *Salmonella* spp. in liquid whole egg (LWE) (approximately pH 7.8) under continuously varying temperature was developed. The model was validated using 2 (5 to 15°C; 600 h and 10 to 40°C; 52 h) sinusoidal, continuously varying temperature profiles. LWE adjusted to pH 7.8 was inoculated with approximately 2.5–3.0 log CFU/mL of *Salmonella* spp., and the growth data at several isothermal conditions (5, 7, 10, 15, 20, 25, 30, 35, 37, 39, 41, 43, 45, and 47°C) was collected. A primary model (Baranyi model) was fitted for each temperature growth data and corresponding maximum growth rates were estimated. Pseudo-R2 values were greater than 0.97 for primary models. Modified Ratkowsky model was used to fit the secondary model. The pseudo-R2 and root mean square error were 0.99 and 0.06 log CFU/mL, respectively, for the secondary model. A dynamic model for the prediction of *Salmonella* spp. growth under varying temperature conditions was developed using 4th-order Runge–Kutta method. The developed dynamic model was validated for 2 sinusoidal temperature profiles, 5 to 15°C (for 600 h) and 10 to 40°C (for 52 h) with corresponding root mean squared error values of 0.28 and 0.23 log CFU/mL, respectively, between predicted and observed *Salmonella* spp. populations. The developed dynamic model can be used to predict the growth of *Salmonella* spp. in LWE under varying temperature conditions.
Practical Application: Liquid egg and egg products are widely used in food processing and in restaurant operations. These products can be contaminated with *Salmonella* spp. during breaking and other unit operations during processing. The raw, liquid egg products are stored under refrigeration prior to pasteurization. However, process deviations can occur such as refrigeration failure, leading to temperature fluctuations above the required temperatures as specified in the critical limits within hazard analysis and critical control point plans for the operations. The processors are required to evaluate the potential growth of *Salmonella* spp. in such products before the product can be used, or further processed. Dynamic predictive models are excellent tools for regulators as well as the processing plant personnel to evaluate the microbiological safety of the product under such conditions.

205. Singh, R., K. M. Cheng, et al. (2009). "Production performance and egg quality of four strains of laying hens kept in conventional cages and floor pens." Poult Sci **88**(2): 256-264. http://www.ncbi.nlm.nih.gov/pubmed/19151338
Production performance and egg quality were compared between 4 strains of beak-trimmed layers: 3 commercial strains-Lohmann White (LW), H&N White (HN), Lohmann Brown (LB)-and a noncommercial cross between Rhode Island Red (male) and Barred Plymouth Rock

(female) in conventional cages and in floor pens. All chicks were reared and 857 pullets were housed at 18 wk of age in their respective environments. Body weight, hen-day egg production, feed consumption and efficiency, and egg quality were measured at wk 20, 30, 40, and 50. In floor pens, the location of eggs was recorded for 4 consecutive days at 4-wk intervals between 20 and 50 wk of age. Eggs from cages, nest-boxes, and the floor were tested for *Escherichia coli* and coliform contamination at 38 and 42 wk of age. Mortality was recorded during the rearing and laying periods. Housing systems significantly influenced BW and mortality but not feed consumption or feed efficiency. The interaction between environment and strain was significant for hen-day egg production at wk 20 to 30 and for BW at wk 30, 40, and 50. Hens in floor pens had greater BW, egg and yolk weights, and yolk color than those in cages. Commercial hens produced more eggs than the cross hens. Overall, HN hens had the best production performance, whereas cross hens had better egg quality. In floor pens, LW and HN hens laid most of their eggs in nest boxes, whereas LB and cross hens laid half of their eggs on the floor. Eggs from cages had lower *E. coli* and coliform contamination than those from nest-boxes and the floor, and *E. coli* contamination was greater for LB eggs than for LW eggs. Significant strain differences were found for the use of nest-boxes, with a high percentage of floor eggs for brown egg strains. This study suggests that genotype x environment interactions should be considered when alternative housing systems are proposed.

206. Sirinavin, S., J. Thavornnunth, et al. (2003). "Norfloxacin and Azithromycin for Treatment of Nontyphoidal *Salmonella* Carriers."
Clin Infect Dis **37**(5): 685-691.
http://cid.oxfordjournals.org/content/37/5/685.full.pdf+html
There has been inadequate evaluation of an antibiotic for eradication of nontyphoidal salmonellae (NTS) in asymptomatic carriers. In a randomized, placebo-controlled trial, such efficacy was evaluated using 2 five-day regimens (norfloxacin, 400 mg twice per day, and azithromycin, 500 mg once per day) compared with placebo. The study included 265 food workers in an area of Thailand where NTS are endemic who were asymptomatic NTS carriers. The presence of NTS in stool samples was assessed on days 7, 30, 60, and 90 after start of treatment. At each assessment visit, <4% of participants in each of the 3 groups carried an initial *Salmonella* serotype; 16%–35% had new *Salmonella* serotypes detected, except on day 7 in the azithromycin group, when the rate was 4%. Sanitation was good at work but not at home. Selection of multidrug-resistant Salmonella enterica serotype Schwarzengrund was demonstrated. The study regimens were not better than placebo for treatment of asymptomatic food workers who carried NTS in an area where these organisms are endemic, and use of the regimens resulted in antimicrobial resistance.

207. Sirsat, S. A., J. K. Choi, et al. (2013). "Persistence of *Salmonella* and *E. coli* on the surface of restaurant menus."
J Environ Health **75**(7): 8-14; quiz 54.
http://www.ncbi.nlm.nih.gov/pubmed/23505769
To the authors' knowledge, the role of restaurant menus as a vehicle for pathogens has not been explored. Menus, however, can pose as a vector for bacterial contamination and transfer. Sampling menus from two restaurants in the Houston, Texas, area showed the presence of up to 100 CFU/cm^2 aerobic bacteria. Follow-up studies designed to investigate the ability of *Salmonella* and *E. coli* to persist on paper and laminated menus at various time points (0, 6, 24, 48, and 72 hours) demonstrated that bacteria persist more efficiently on laminated menus as compared to paper menus. Transfer studies performed to quantitatively determine the ability of bacteria to transfer from menus to fingertips and from fingertips to clean menus showed that bacteria can be transferred for up to 24 hours. The study described here showed that restaurant menus may serve as vehicles for pathogens and hence present a public health issue within the retail food environment.

208. Slader, J., G. Domingue, et al. (2002). "Impact of transport crate reuse and of catching and processing on *Campylobacter* and *Salmonella* contamination of broiler chickens."
Appl Environ Microbiol **68**(2): 713-719.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC126660/pdf/0103.pdf
The influence of transport, catching, and processing on contamination of broiler chickens with *Salmonella* and *Campylobacter* was investigated. Transport crates were reused with high

frequency and were often still contaminated with *Salmonella* and *Campylobacter* when they arrived at the farm despite the fact that they were washed at the factory, and thus they were a potential route of infection. These organisms contaminated the feathers of previously *Campylobacter* and *Salmonella*-negative birds going to the processing plant and were isolated from processed carcasses, albeit at a low frequency. The *Campylobacter* types which were the predominant organisms on the live birds when they arrived at the processing plant were not necessarily the types that were most frequently isolated from processed carcasses. This finding may reflect cross-contamination that occurred during processing or differences in the tolerance of the strains to the hostile environments that the bacteria experienced. The process of catching and putting the birds in crates significantly increased the chance of contamination with *Campylobacter* ($P < 0.001$).

209. Smeltzer, T. I., K. Orange, et al. (1979). "Bacterial penetration in floor and nest box eggs from meat and layer birds."
Aust Vet J **55**(12): 592-593.
http://www.ncbi.nlm.nih.gov/pubmed/556359
A method similar to that used by Board and Board (1967) was used to determine the numbers of eggs penetrated by bacteria on 3 poultry farms in south-east Queensland. Significant differences in the percentages of penetrated eggs between the eggs of layer birds (9.7%) and the eggs of meat birds (16.1%) and between nest eggs (10.5%) and floor eggs (15.3%) were detected. The distribution of the numbers of penetration points was similar for nest and floor eggs for both types of bird and was independent of shell surface area or thickness.

210. Steenackers, H., K. Hermans, et al. (2012). "*Salmonella* biofilms: An overview on occurrence, structure, regulation and eradication."
Food Res Int **45**(2): 502-531.
http://www.sciencedirect.com/science/article/pii/S0963996911000615
The ability of *Salmonella* to form complex surface-associated communities, called biofilms, contributes to its resistance and persistence in both host and non-host environments and is especially important in food processing environments. In this review, the different types of abiotic (plastic, glass, cement, rubber, and stainless steel) and biotic surfaces (plant surfaces, epithelial cells, and gallstones) on which *Salmonella* biofilms have been described are discussed, as well as a number of commonly used laboratory setups to study *Salmonella* biofilm formation (rdar morphotype, pellicle formation, and biofilms on polystyrene pegs). Furthermore, the structural components important during *Salmonella* biofilm formation are described (curli and other fimbriae, BapA, flagella, cellulose, colanic acid, anionic O-antigen capsule and fatty acids), with special attention to the structural variations of biofilms grown on different surfaces and under different conditions. Indeed, biofilm formation is strongly influenced by different environmental signals, via a complex regulatory network. An extensive overview is given on the current understanding of this genetic network and the interactions between its different components (CsgD, RpoS, Crl, OmpR, IHF, H-NS, CpxR, MlrA, c-di-GMP, BarA/SirA, Csr, PhoPQ, RstA, Rcs, metabolic processes and quorum sensing). To further illustrate that biofilm formation is a mechanism of *Salmonella* to adapt to different environments, the resistance of *Salmonella* biofilms against different stress factors including desiccation stress, disinfectants (e.g. hypochlorite, glutaraldehyde, cationic tensides and triclosan) and antibiotics (e.g. ciprofloxacin) is described. Finally, a number of Salmonella biofilm inhibitors, identified through bottom-up- and top-down-approaches, are discussed, such as surfactin, glucose, halogenated furanones, 4(5)-aryl 2-aminoimidazoles, furocoumarins and salicylates. Also the potential of combination therapy (e.g. combinations of triclosan and quaternary ammonium salts or halogenated furanones and antibiotics/disinfectants) and nano- and micro-emulsions to inhibit *Salmonella* biofilm formation is discussed. Insight into the pathogen's complex biofilm process will eventually lead to further unraveling of its intricacies and more efficient strategies to combat *Salmonella* biofilms.

211. Stepanović, S., I. Ćirković, et al. (2004). "Biofilm formation by *Salmonella* spp. and *Listeria monocytogenes* on plastic surface."
Lett Appl Microbiol **38**(5): 428-432.
http://onlinelibrary.wiley.com/doi/10.1111/j.1472-765X.2004.01513.x/full

Aims: To investigate the biofilm formation by 122 *Salmonella* spp. and 48 *Listeria monocytogenes* strains on a plastic surface.
Methods: Quantification of biofilm formation was performed in brain heart infusion (BHI), trypcase soya broth (TSB), meat broth (MB) and 1/20 diluted trypcase soya broth (1/20-TSB) in plastic microtitre plates.
Results: All tested *Salmonella* spp. and *L. monocytogenes* strains produced biofilm in a suitable medium. However, the quantities of biofilm produced by *Salmonella* spp. were greater than those produced by tested *L. monocytogenes* strains. The nutrient content of the medium significantly influenced the quantity of produced biofilm. Diluted TSB was the most effective in promoting biofilm production by *Salmonella* spp., followed by TSB, while the least quantity of biofilm was formed in BHI and MB. *L. monocytogenes* produced the highest quantities of biofilm in BHI, followed by TSA, then MB, and the least quantities of biofilm were produced in 1/20-TSB.
Conclusions:*Salmonella* spp. produces more biofilm in nutrient-poor medium, while *L. monocytogenes* produce more biofilm in nutrient-rich medium.

212. Stephens, N., D. Coleman, et al. (2008). "Recurring outbreaks of *Salmonella* Typhimurium phage type 135 associated with the consumption of products containing raw egg in Tasmania."
Commun Dis Intell Q Rep **32**(4): 466-468.
http://www.ncbi.nlm.nih.gov/pubmed/19374277
Large egg-associated outbreaks of *Salmonella* Typhimurium 135 (STm135) that were associated with inadequate food safety practices but also linked to a common poultry farm occurred in Tasmania in 2005. A series of public health interventions were implemented to prevent further occurrences but 2 more egg-associated outbreaks in Tasmania in March 2007 and January 2008 led to a further 66 cases of STm135. This report describes these outbreaks and their links to the common source associated with the outbreaks in 2005.

213. Stephens, N., C. Sault, et al. (2007). "Large outbreaks of *Salmonella* Typhimurium phage type 135 infections associated with the consumption of products containing raw egg in Tasmania."
Commun Dis Intell Q Rep **31**(1): 118-124.
http://www.ncbi.nlm.nih.gov/pubmed/17503652
This report describes one of the largest egg-associated outbreaks of foodborne illness in Australia for many years. Between June and December 2005, five outbreaks of *Salmonella* Typhimurium phage type 135 were identified in Tasmania, leading to 125 laboratory-confirmed cases. Public health investigations included case and food handler interviews, cohort studies, environmental health investigations of food businesses, microbiological testing, traceback, and inspections and drag swabbing of an egg farm. These investigations enabled identification of foods containing raw egg or foods contaminated through inadequate food handling and/or storage procedures as possible vehicles for infection. A particular poultry farm was reported as the common source of eggs. Interventions targeting the general public and food handlers to promote better handling of egg products, and advice to egg producers regarding harm minimisation strategies led to the series of outbreaks being brought under control.

214. Stocki, S. L., C. B. Annett, et al. (2007). "Persistence of *Salmonella* on egg conveyor belts is dependent on the belt type but not on the rdar morphotype."
Poult Sci **86**(11): 2375-2383.
http://ps.oxfordjournals.org/content/86/11/2375.long
Commercial caged layer flocks in Alberta, Canada, are commonly monitored for *Salmonella enterica* serovar Enteritidis (SE) and *S. enterica* serovar Typhimurium (ST) by environmental sampling. In one recent case, a SE strain isolated from the egg conveyor belt was a source of persistent infection for the flock. This study was undertaken to examine *Salmonella* colonization on egg conveyor belts and to determine whether the rdar morphotype, a conserved physiology associated with aggregation and long-term survival, contributed to persistence. Four woven belts constructed of natural or nonnatural fibers and a 1-piece belt made of vinyl were tested with rdar-positive ST and SE strains and a rdar-negative ST DeltaagfD reference strain. The type of egg belt was the most important factor influencing *Salmonella* colonization and persistence. The vinyl belt, with the least surface area available

for colonization, had the fewest *Salmonella* remaining after washing and disinfection, whereas the hemp-plastic belt, with the greatest surface area, had the most *Salmonella* remaining. Real-time gene expression indicated that the rdar morphotype was involved in colonizing the egg belt pieces; however, it was not essential for persistence. In addition, rdar-positive and rdar-negative strains were equally similarly to disinfection on the egg belt pieces. The results indicate that *Salmonella* can persist on a variety of egg belts by mechanisms other than the rdar morphotype, and that using egg conveyer belts with reduced surface area for bacterial colonization can lessen contamination problems.

215. Stokes, J. L., W. W. Osbourne, et al. (1956). "Penetration and growth of *Salmonella* in shell eggs."
J Food Sci **21**(5): 510-518
http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.1956.tb16950.x/abstract
There are several ways in which shell eggs can become contaminated externally and internally, with *Salmonella*. Normally, the oviduct of the hen is sterile and therefore the shell and internal contents of the egg are also free of microorganisms. In some instances, however, the ovaries and oviduct may be infected with *Salmonella* and these may be deposited inside the egg. More frequently, however, the egg becomes contaminated after it is laid. *Salmonella*, are deposited on the shell from the intestinal tract of the lien and from other sources in the environment. In one survey, 2% of clean eggs and 16% of dirty eggs were found to contain *Salmonella* on the surface or in the pores of the shell. Shell contamination is undoubtedly a major source of *Salmonella* in dried and frozen liquid whole egg, yolk, and albumen. Moreover, these percentages of *Salmonella* are minimal figures due to inaccuracies inherent in the isolation and identification procedures.

216. Stringfellow, K., P. Anderson, et al. (2009). "Evaluation of disinfectants commonly used by the commercial poultry industry under simulated field conditions."
Poult Sci **88**(6): 1151-1155.
http://ps.oxfordjournals.org/content/88/6/1151.full.pdf
The correct usage of disinfectants is an important component of a successful biosecurity program. The objective of this study was to determine the effect of time, temperature, and organic matter (OM) on disinfectant efficacy. *Staphylococcus aureus* and *Salmonella* Typhimurium were used to represent gram-negative and gram-positive bacteria commonly found in commercial poultry housing. The first study evaluated the effect of temperature (4, 20, 32, or 43 degrees C) and time (1, 2, 3, 4, 6, 8, 12, 16, 20, 24, and 30 wk) on the efficacy of disinfectants diluted to working concentrations. The second study determined the effect of OM on the efficacy of working concentrations of freshly prepared disinfectants against the bacteria. For the third study, we compared the bactericidal properties of freshly prepared disinfectants and 30-wk-old disinfectants in the presence of OM. Quaternary ammonium-, chlorhexidine-, phenolic-, and binary ammonium-based solutions represented disinfectants commonly used within the poultry industry. In the first study, all of the disinfectants were effective against *S. aureus* and *Salmonella* Typhimurium regardless of treatment. However, the phenolic compound had reduced (P <or= 0.05) efficacy against *Salmonella* Typhimurium after 6 wk of storage at the highest temperature of 43 degrees C and after 16 wk at the second highest temperature of 32 degrees C. All of the disinfectants were effective against *S. aureus* regardless of temperature treatment. In the second study, the addition of sterile chicken litter had deleterious effects on all 4 classes of disinfectants against *Salmonella* Typhimurium. Of the disinfectants tested, the phenolic compound retained efficacy against *S. aureus*. In the third study, the presence of OM significantly reduced (P <or= 0.05) the efficacy of the 30-wk-old quaternary ammonium and phenolic compound against *Salmonella*. The fresh quaternary ammonium and binary compound achieved a greater kill (P <or= 0.05) of *Staphylococcus*, relative to the 30-wk-old disinfectant. These results emphasize the need to use fresh disinfectants and that OM should be removed before disinfection.

217. Sumner, J., G. Raven, et al. (2003). "Which food categories cause salmonellosis in Australia?"
Food Aust **55**(12): 597-601
Using data on salmonellosis and *Salmonella* isolations from foods and animals gathered by the National Enteric Pathogens Surveillance Scheme (NEPSS), an attempt has been made, by serovar matching, to quantify the involvement of different food commodities in

salmonellosis. Based on NEPSS data from 2000, it is suggested that poultry and egg products were associated with 35% of salmonelloses, meat products with 31%, plant foods with 10% and the remainder (24%) with Other food categories. Serovar matching from the same primary data sources also implicates pet animals as a possible source of salmonellosis. A need is indicated for more proactive links between food testing and controlling authorities in order to reverse current upwards trends in food poisoning in Australia.

218. Sumner, J., G. Raven, et al. (2004). "Have changes to meat and poultry food safety regulation in Australia affected the prevalence of *Salmonella* or of salmonellosis?"
Int J Food Microbiol **92**(2): 199-205.
http://www.ncbi.nlm.nih.gov/pubmed/15109797
During the 1990s, there was radical change in regulation of meat and poultry hygiene in Australia, and Australian Standards were developed for each sector of the meat industry. Systems for industry/government co-regulation and company-employed meat inspection were introduced based on company HACCP programs approved and audited by the Controlling Authority. However, in the 5 years since regulatory changes took full effect, rates of salmonellosis have not decreased (surveillance and reporting systems have remained unchanged). Using statistics gathered by the National Enteric Pathogens Surveillance Scheme, an attempt was made to link *Salmonella* serovars isolated from meat and poultry with those causing salmonellosis. Two periods were studied, 1993/1994, before regulations were introduced, and 2000/2001, when regulations should be having an effect. For red meat, the same serovars were prominent among the top 10 isolates both before and after regulation, and there was little linkage with salmonelloses. For poultry, frequently isolated serovars differed pre- and post-regulation, however, in both periods there was some linkage between serovars isolated from poultry and those causing salmonelloses. Using published and unpublished survey data, it was concluded that there had been improvements in microbiological quality of red meat and poultry over the same timeframe as regulatory changes. That these improvements apparently have not carried through to reduced case-rates for salmonellosis may be due to numerous causes, including lack of control in the food processing, food service and home sectors. The present paper illustrates difficulties faced by governments in measuring public health outcomes of changes to food hygiene regulation.

219. Swanson, S. J., C. Snider, et al. (2007). "Multidrug-Resistant *Salmonella* enterica Serotype Typhimurium Associated with Pet Rodents."
N Engl J Med **356**(1): 21-28.
http://www.nejm.org/doi/pdf/10.1056/NEJMoa060465

220. Takase, K., T. Nakayama, et al. (1999). "Growth of *Salmonella* Typhimurium and *Salmonella* Enteritidis in Egg Yolks from Highly Immunized Hens."
J Vet Med Sci **61**(8): 959-960
This experiment was conducted to ascertain whether the growth of *Salmonella* Enteritidis (SE) or *Salmonella* Typhimurium (ST) would be suppressed in the presence of antibodies contained in egg yolks. Specific pathogen-free chickens (102 days of age) were subcutaneously immunized with oil-adjuvanted bacterin of SE or ST, twice within a four-week interval. During 160 to 170 days of age, eggs were collected, the yolks were removed and mixed with an equal volume of physiological buffered saline, inoculated with ten colony forming units (CFU) of SE or ST, and incubated at 37°C or 20°C for 23 hr. The growth of organisms in each yolk solution was examined. The egg yolk derived from non-immunized hens was examined in the same manner as the controls. There was no difference in the growth titer between the antibody-positive yolk and the negative yolk. The result suggests that the antibodies in the yolk do not influence the growth of each organism, even if the hens are highly immunized.

221. Taormina, P. J. and W. J. Dorsa (2007). "Evaluation of hot-water and sanitizer dip treatments of knives contaminated with bacteria and meat residue."
J Food Prot **70**(3): 648-654.
http://www.ncbi.nlm.nih.gov/pubmed/17388054
Hot water (HW; 82.2 degrees C, 180 degreesF) is used for sanitation of meat cutting implements in most slaughter facilities, but validation of actual practices against meat-borne bacterial pathogens and spoilage flora is lacking. Observed implement immersions in HW in

two large pork processing plants were found to typically be < or = 1 s. Impact of these practices on bacteria on metal surfaces was assessed in the laboratory, and alternative treatments were investigated. Knives were inoculated with raw pork residues and *Escherichia coli* O157:H7, *Salmonella* Typhimurium DT104, *Clostridium perfringens*, and *Lactobacillus* spp. and were sampled before and after 1- or 15-s dips of blades in HW, warm water (48.9 degrees C), or warm sanitizers (neutral or acid quaternary ammonium compounds [QAC] at 400 ppm, or peroxyacetic acid at 700 ppm H2O2 and 165 ppm peroxyacetic acid). Simultaneous scrubbing and 15-s dipping in HW or acid QAC was also evaluated. Reductions on knives dipped for 1 s were usually < 1 log and were not significantly different (P > 0.05) between treatments. Reductions of *E. coli* O157:H7 after 15 s in HW, neutral QAC, acid QAC, or peroxyacetic acid were 3.02, 2.38, 3.04, and 1.52 log, respectively. Reductions of other bacteria due to HW were not significantly different from sanitizers and were significantly greater than warm water for all bacteria except C. perfringens. Combined scrubbing and 15-s dipping in HW resulted in a 2.91- and 2.25-log reduction of *E. coli* O157:H7 and *Salmonella* Typhimurium DT104, respectively, whereas reduction caused by acid QAC was significantly less at about 1.7 log each. Brief dip treatments of contaminated knives have limited efficacy, but longer immersions cause greater reductions that were not enhanced by scrubbing. QAC is a suitable alternative to HW in this application.

222. Thomas, C., B. Daughtry, et al. (2006). An Egg: *Salmonella* quantitative risk assessment model.
AECL Project SAR-42A.
This project aimed to conduct a quantitative risk assessment of *Salmonella* contamination of eggs in Australia. A key outcome was the development of a tool to predict how changes in industry practices may impact on the occurrence of Salmonellosis in humans. The risk assessment process provides a scientifically objective process for identifying risk and appropriate options for control. The approach is increasingly used to develop public health standards and as a basis for international market access. The report provides a comprehensive review of food-borne outbreaks in which eggs along with other ingredients were implicated (Hazard Identification). These outbreaks were evaluated in terms of the level of certainty of attribution to eggs. The occurrence and serovar of *Salmonella* contaminants from layer flocks, unpasteurised and pasteurised egg products were compared with serovars implicated in outbreaks potentially attributable to eggs. The source of eggs, type of foods, settings of outbreaks and populations exposed were evaluated to define the circumstances in which eggs may be the source of contamination. Such information serves as a guide for the implementation of appropriate controls from farm to consumption. To gain an estimate of the level of egg contamination (Exposure Assessment), a pilot survey of *Salmonella* prevalence on ungraded (off-farm), graded/washed and contents of graded eggs produced in cages was conducted. In addition, the external contamination of barn laid and free-range eggs was established. This data was used to develop a modular exposure assessment model for each stage of production from point of lay, collection, storage, transportation and processing to the end of retail storage. A survey of egg production and processing practices in Australia was used to obtain necessary data representative of the Australian layer industry practices. The modular approach to design of the Exposure Assessment was similar to previously published Exposure Assessments (FAO-WHO, 2002 and USDA-FSIS, 1998). Risk of Salmonellosis to consumers was estimated for foods prepared with three different cooking effects and is expressed in terms of probability of illness per million servings under Australian conditions to model options for reduction of consumer risk. An exposure assessment and risk characterisation for egg products was also undertaken.

223. Todd, E. C. (1996). "Risk assessment of use of cracked eggs in Canada."
Int J Food Microbiol **30**(1-2): 125-143.
http://www.ncbi.nlm.nih.gov/pubmed/8856379
In 1992, Agriculture and Agri-Food Canada (AAFC) amended its Egg Regulations to restrict movement of Canada C eggs (cracks) to federally registered processed egg stations for pasteurization. This was questioned by egg producers and some provinces on economic grounds. It was also in conflict with long-standing practices of marketing eggs in some provinces to retail stores, bakeries, restaurants and institutions or at the farm gate. In order to determine how much of a risk these eggs were to human health, AAFC requested that the Health Protection Branch (HPB) of Health Canada (HC) conduct a risk assessment. On the

basis of outbreak data, the main hazard in these eggs was identified as *Salmonella*. Salmonellae may occasionally be present on shell eggs even after washing, and any *Salmonella* reaching the membranes can be transferred to an egg mixture through breaking, and will rapidly grow under improper storage conditions. A Relative Risk analysis showed that cracked eggs are 3 to 93 times more likely than uncracked shell eggs to cause outbreaks. A probability of illness of 1 in 3800 was derived from the 40 million cracked eggs produced in Canada and not pasteurized and the probable 10,500 illnesses arising from these. This was for the general population, but this would be greater for those who consume many shell eggs or would do so in an unsafe manner, or are more likely to be infected (5% of consumers who eat raw or lightly cooked eggs daily, rural communities with more opportunities for obtaining cracked eggs, and those who are immunocompromised and in institutions). Even though it is not possible to precisely determine the risk of salmonellosis through cracked eggs, this assessment indicated that there was enough of a concern that a management strategy was needed. Eight options for managing the risk were considered and ranked for acceptability by both HC and AAFC. Ideally, all cracked eggs should be broken and pasteurized, but this is impractical in certain regions of the country, and other options, such as sales to food processors operating under Good Manufacturing Practices (GMP), and at the farm gate in marked cartons and under controlled conditions, were considered to be acceptable, whereas sales to institutions and bakeries were not. This is the first fromal food-related microbiological risk assessment that HC has completed. Although this is a Canadian problem, any country producing eggs has to recognise that despite any regulations controlling the use of cracked eggs, economics will dictate that some of these will be consumed as whole eggs or egg products, and a management plan is desirable to limit hazardous practices associated with these eggs.

224. Trampel, D. W., T. G. Holder, et al. (2014). "Integrated farm management to prevent *Salmonella* Enteritidis contamination of eggs."
J Appl Poult Res **23**:353-365.
http://japr.oxfordjournals.org/content/23/2/353.full.pdf
Salmonella Enteritidis in contaminated eggs is a public health hazard that may cause hospitalization or death in the elderly, infants, and individuals with impaired immune systems. Prevention of *Salmonella* Enteritidis infection of laying hens is an essential first step in reducing *Salmonella* Enteritidis outbreaks in humans. Multiple interventions at several stages during egg production can combine to reduce numbers of infected chickens and keep egg contamination to low levels. Every effort should be made to exclude *Salmonella* Enteritidis from egg production premises by implementing effective biosecurity measures, stocking the farm with *Salmonella* Enteritidis-free replacement pullets, controlling rodent and insect vectors, and denying wild birds and pets access to chicken houses. Diligent cleaning and disinfection of chicken houses before introduction of a new flock will minimize environmental exposure and indirect horizontal transmission of multiple pathogens, including *Salmonella* Enteritidis. Increased resistance of chickens to intestinal colonization by *Salmonella* Enteritidis can be attained by the use of probiotics, prebiotics, and synbiotics. Laying hens should be immunized with live and killed vaccines to stimulate mucosal and systemic immunity and reduce the prevalence of *Salmonella* Enteritidis-contaminated eggs. Shell eggs should be refrigerated as soon as possible after laying to keep *Salmonella* Enteritidis cells at low levels in any contaminated eggs. Comprehensive *Salmonella* Enteritidis-control programs have proven to be successful in reducing the incidence of *Salmonella* Enteritidis infections in both egg-laying flocks and humans.

225. Umali, D. V., R. R. Lapuz, et al. (2012). "Transmission and shedding patterns of *Salmonella* in naturally infected captive wild roof rats (Rattus rattus) from a *Salmonella*-contaminated layer farm."
Avian Dis **56**(2): 288-294.
http://www.ncbi.nlm.nih.gov/pubmed/22856184
Rodents play a major role in the transmission and maintenance of *Salmonella* contamination cycles in poultry facilities. However, very limited field data are available regarding the transmission routes, infection cycle, and shedding patterns of *Salmonella* by naturally infected wild rodents from commercial layer farms. In this study, a total of 128 resident wild roof rats (Rattus ratus) were captured from a *Salmonella*-contaminated layer facility. All roof rats were divided into 51 laboratory cages, and weekly monitoring of *Salmonella* fecal shedding

patterns was conducted for 53 wk. Seven roof rats from cages that were observed to frequently shed *Salmonella* were isolated in individual cages, and daily *Salmonella* monitoring was performed for 35 days. At the end of monitoring, each roof rat was euthanatized, and isolation of *Salmonella* from different organs was performed. Results of weekly monitoring of *Salmonella* showed that 21 of 51 cages (41.2%) were positive for *Salmonella* Infantis, while two cages (3.92%) were positive for *Salmonella* Enteritidis. Moreover, 11 cages were positive for *Salmonella* for at least two sampling weeks. Isolation of *Salmonella* from fecal droppings was mainly observed during the first 12 wk of captivity. The longest interval between two *Salmonella*-positive fecal dropping was 24 wk. In the daily *Salmonella* monitoring, only *Salmonella* Infantis was isolated from fecal droppings, in which the highest number of *Salmonella* Infantis organisms per fecal dropping was at 1 x 10(8) colony-forming units (cfu), while the lowest measured quantity was 1 x 10(3) cfu. It was noted that the frequency of *Salmonella* shedding in fecal droppings appeared to have a linear correlation (r = 0.85) with the number of *Salmonella* organisms (cfu) per fecal pellet (P < 0.05). Moreover, pulsed-field gel electrophoresis analysis of *Salmonella* Infantis isolates revealed a single identical pulsed-field pattern. *Salmonella* Enteritidis isolates from fecal droppings and internal organs also generated a single identical pulsed-field pattern. Interestingly, *Salmonella* Infantis was not isolated from any of the organs examined, while *Salmonella* Enteritidis was isolated from the spleen and liver of one roof rat. These results may indicate that wild roof rats could persistently carry *Salmonella* and contaminate commercial poultry facilities through intermittent fecal shedding. Moreover, *Salmonella* Enteritidis in wild roof rats appears to be more of a systemic infection, in which isolation is most likely to occur in internal organs, whereas *Salmonella* Infantis is more likely an enteric type of infection, in which isolation is most likely to occur in the intestinal contents. It is very plausible that layer chickens could become infected with *Salmonella* through ingestion of *Salmonella*-positive fecal droppings or feeds contaminated with these fecal droppings from infected resident roof rats. This is likely one of the major reasons why layer houses can be persistently infected by *Salmonella* even if the facilities are thoroughly cleaned and disinfected and if replacement stocks are obtained from *Salmonella*-free breeders and rearing units. It is therefore a noteworthy suggestion that rodent control programs inside poultry premises comprise an essential and effective tool in the management and control of *Salmonella* contamination in layer flocks.

226. Upadhyaya, I., A. Upadhyay, et al. (2013). "Rapid inactivation of *Salmonella* Enteritidis on shell eggs by plant-derived antimicrobials."
Poult Sci **92**(12): 3228-3235.
http://www.ncbi.nlm.nih.gov/pubmed/24235233
Salmonella Enteritidis is a common foodborne pathogen transmitted to humans largely by consumption of contaminated eggs. The external surface of eggs becomes contaminated with *Salmonella* Enteritidis from various sources on farms, the main sources being hens' droppings and contaminated litter. Therefore, effective egg surface disinfection is critical to reduce pathogens on eggs and potentially control egg-borne disease outbreaks. This study investigated the efficacy of GRAS (generally recognized as safe) status, plant-derived antimicrobials (PDA), namely trans-cinnamaldehyde (TC), carvacrol (CR), and eugenol (EUG), as an antimicrobial wash for rapidly killing *Salmonella* Enteritidis on shell eggs in the presence or absence of chicken droppings. White-shelled eggs inoculated with a 5-strain mixture of nalidixic acid (NA) resistant *Salmonella* Enteritidis (8.0 log cfu/mL) were washed in sterile deionized water containing each PDA (0.0, 0.25, 0.5, or 0.75%) or chlorine (200 mg/kg) at 32 or 42 degrees C for 30 s, 3 min, or 5 min. Approximately 6.0 log cfu/mL of *Salmonella* Enteritidis was recovered from inoculated and unwashed eggs. The wash water control and chlorine control decreased *Salmonella* Enteritidis on eggs by only 2.0 log cfu/mL even after washing for 5 min. The PDA were highly effective in killing *Salmonella* Enteritidis on eggs compared with controls (P < 0.05). All treatments containing CR and EUG reduced *Salmonella* Enteritidis to undetectable levels as rapidly as within 30 s of washing, whereas TC (0.75%) completely inactivated *Salmonella* Enteritidis on eggs washed at 42 degrees C for 30 s (P < 0.05). No *Salmonella* Enteritidis was detected in any PDA or chlorine wash solution; however, substantial pathogen populations (~4.0 log cfu/mL) survived in the antibacterial-free control wash water (P < 0.05). The CR and EUG were also able to eliminate *Salmonella* Enteritidis on eggs to undetectable levels in the presence of 3% chicken droppings at 32 degrees C (P < 0.05). This study demonstrates that PDA could effectively be used as a wash

treatment to reduce *Salmonella* Enteritidis on shell eggs. Sensory and quality studies of PDA-washed eggs need to be conducted before recommending their use.

227. Upadhyaya, S. K., J. R. Cooke, et al. (1985). "A fluid-filled spherical shell model of the thermo-elastic behaviour of avian eggs."
J Agric Eng Res **32**(2): 95-109.
http://www.sciencedirect.com/science/article/pii/0021863485900708
Egg shell breakage during hot water washing results from the differences in the volumetric expansion characteristics of the shell and its contents. Upon heating, the yolk and albumen expand while the enclosed volume of a hollow egg shell has an anomolous shrinkage response. An incompressible liquid and compressible gas-filled elastic, spherical shell model is developed to examine the thermoelastic response to hot water washing. Two bounding cases of a shell which is (1) completely impervious and (2) completely porous to air cell leakage are considered. Egg shell strength is known to decrease with increasing temperature. When combined with internal pressures induced by the washing process, a temporary, but appreciable reduction in the shell's capacity to withstand mechanical loads is predicted. Increases in shell porosity, air cell volume and egg shell size decrease the thermally produced mechanical stress. On the other hand, shell thickness does not appreciably influence the thermally induced stress.

228. Vadehra, D. V., R. C. Baker, et al. (1969). "*Salmonella* Infection of Cracked Eggs."
Poult Sci **48**(3): 954-957.
http://ps.oxfordjournals.org/content/48/3/954.full.pdf+html
Little work has been done on the susceptibility of cracked eggs to infection and spoilage by microorganisms, as compared to the extensive literature of spoilage of normal shell eggs. Cracked eggs constitute about 2–3% of total egg production and are of great economic importance to the poultry industry. With the recent reports of occurrence of *Salmonella* in egg and egg products, the safety of cracked eggs and the products produced from them for human consumption has been questioned by public health authorities and others. Previous work done by McNally (1953) indicated that temperature and humidity changes affect cracked eggs adversely as compared to normal shell eggs. Brown et al. (1966) working with *Pseudomonas aeruginosa* showed that bacterial numbers in the dip water were more important than a temperature differential for infection. They also reported that cracked eggs were no more susceptible to infection by *P. aeruginosa* than uncracked eggs as long as they were handled properly. This study was carried out to determine the relative susceptibility of cracked and normal shell eggs to invasion by *Salmonella* organisms. The effect of the presence of several chemicals in the wash water on the spoilage of cracked and normal shell eggs was also investigated.

229. Van Immerseel, F., U. Methner, et al. (2005). "Vaccination and early protection against non-host-specific *Salmonella* serotypes in poultry: exploitation of innate immunity and microbial activity."
Epidemiol Infect **133**(6): 959-978.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2870330/pdf/16274493.pdf
A recent European Union Directive required member states to put monitoring and control programmes in place, of which vaccination is a central component. Live *Salmonella* vaccines generally confer better protection than killed vaccines, because the former stimulate both cell-mediated and humoral immunity. Administering *Salmonella* bacteria orally to newly hatched chickens results in extensive gut colonization and a strong adaptive immune stimulus but broiler chickens are immunologically immature. However, colonization exerts a variety of rapid (within 24 h) protective effects. These include specific colonization-inhibition (competitive exclusion) in which the protective bacteria exert a profound resistance to establishment and colonization by other related bacteria. This is thought to be primarily a metabolic attribute of the vaccinating bacteria but may also involve competition for attachment sites. The presence of large numbers of bacteria originating from a live *Salmonella* vaccine in the intestine can also induce infiltration of polymorphonuclear cells into the intestinal wall, which confers resistance to invasion and systemic spread by virulent *Salmonella* strains. This opens new perspectives for vaccine usage in broilers, layers and breeding poultry but also in other animals which show increased susceptibility to infection because of their young age or for other reasons, such as oral chemoprophylaxis or chemotherapy, where the lack of

established normal gut flora is an issue. We recommend that all live vaccines considered for oral administration should be tested for their ability to induce the two protective effects described above. Further developments in live *Salmonella* vaccines are, however, currently hindered by fears associated with the use and release of live vaccines which may be genetically modified.

230. Van Immerseel, F., J. B. Russell, et al. (2006). "The use of organic acids to combat *Salmonella* in poultry: a mechanistic explanation of the efficacy."
Avian Pathol **35**(3): 182-188.
http://www.tandfonline.com/doi/full/10.1080/03079450600711045#abstract
Salmonella is a human pathogen that is commonly found in poultry products. It is possible to decrease chicken carcass and egg contaminations by adding organic acids to the feed or drinking water at appropriate times. Medium-chain fatty acids are more antibacterial against *Salmonella* than short-chain fatty acids. The antibacterial effect of these acids is species specific. Bacteria that are unable to decrease intracellular pH accumulate organic acid anions in accordance with the pH gradient across their cell membranes. The short-chain fatty acid butyrate specifically down-regulates expression of invasion genes in *Salmonella* spp. at low doses. Also medium-chain fatty acids and propionate decrease the ability of *Salmonella* spp. to invade epithelial cells, in contrast to acetic acid. Because not all bacteria are affected in a similar fashion by organic acids, it may be possible to use probiotic and prebiotic bacteria to achieve beneficial effects. If diets can be designed to stimulate organic acid production in the caecum, it may be possible to control *Salmonella* spp. via even easier and more cost-effective measures, compared with addition of acids to feed or drinking water.

231. Vanselow, B. A., M. A. Hornitzky, et al. (2007). "*Salmonella* and on-farm risk factors in healthy slaughter-age cattle and sheep in eastern Australia."
Aust Vet J **85**(12): 498-502.
http://www.ncbi.nlm.nih.gov/pubmed/18042157
OBJECTIVE: To examine healthy slaughter-age cattle and sheep on-farm for the excretion of *Salmonella* serovars in faeces and to identify possible risk factors using a questionnaire. PROCEDURE: The study involved 215 herds and flocks in the four eastern states of Australia, 56 with prior history of salmonellosis. Production systems examined included pasture beef cattle, feedlot beef cattle, dairy cattle, prime lambs and mutton sheep and animals were all at slaughter age. From each herd or flock, 25 animals were sampled and the samples pooled for *Salmonella* culture. All *Salmonella* isolated were serotyped and any *Salmonella* Typhimurium isolates were phage typed. Questionnaires on each production system, prepared in Epi Info 6.04, were designed to identify risk factors associated with *Salmonella* spp excretion, with separate questionnaires designed for each production system. RESULTS: Salmonellae were identified in all production systems and were more commonly isolated from dairies and beef feedlots than other systems. Statistical analysis revealed that dairy cattle were significantly more likely to shed *Salmonella* in faeces than pasture beef cattle, mutton sheep and prime lambs ($P<0.05$). A wide diversity of *Salmonella* serovars, all of which have been isolated from humans in Australia, was identified in both cattle and sheep. Analysis of the questionnaires showed access to new arrivals was a significant risk factor for *Salmonella* excretion on dairy properties. For beef feedlots, the presence of large numbers of flies in the feedlot pens or around stored manure were significant risk factors for Salmonella excretion.
CONCLUSION: Dairy cattle pose the highest risk of all the slaughter-age animals tested. Some of the identified risk factors can be overcome by improved management practices, especially in relation to hygiene.

232. Veldman, A., H. A. Vahl, et al. (1995). "A survey of the incidence of *Salmonella* species and *Enterobacteriaceae* in poultry feeds and feed components."
Vet Rec **136**(7): 169-172.
http://www.ncbi.nlm.nih.gov/pubmed/7762127
Between July 1990 and April 1991 the rate of contamination with *Salmonella* species of poultry feeds and feed components used by the Dutch feed industry was surveyed. Ten per cent of 360, 10 g samples of poultry feeds were found to be contaminated. Mash feeds, mostly used for layer-breeders, were far more frequently (21 per cent) contaminated than pelleted feeds (1.4 per cent). The rate of contamination of 130 samples of fish meal was 31

per cent, of 83 samples of meat and bone meal 4 per cent, 58 samples of tapioca 2 per cent and of 15 samples of maize grits 27 per cent. Twenty-eight serotypes of salmonellae were isolated, but no *Salmonella* Enteritidis was found, despite the occurrence of an epidemic in poultry caused by this serotype since 1987. The serotypes isolated most frequently were not the same as those encountered in poultry flocks. The *Enterobacteriaceae* isolated from the feedstuffs were predominantly thermotrophic. They were shown to be useful markers of the rate of contamination with salmonellae and of the efficiency of decontamination of the feedstuffs by pelletisation.

233. Veluz, G. A., S. Pitchiah, et al. (2012). "Attachment of *Salmonella* serovars and *Listeria monocytogenes* to stainless steel and plastic conveyor belts."
Poult Sci **91**(8): 2004-2010.
http://ps.oxfordjournals.org/content/91/8/2004.long
In poultry industry, cross-contamination due to processing equipment and contact surfaces is very common. This study examined the extent of bacterial attachment to 6 different types and design of conveyor belts: stainless steel-single loop, stainless steel-balance weave, polyurethane with mono-polyester fabric, acetal, polypropylene mesh top, and polypropylene. Clean conveyor belts were immersed separately in either a cocktail of Salmonella serovars (*Salmonella* Typhimurium and *Salmonella* Enteritidis) or *Listeria monocytogenes* strains (Scott A, Brie 1, ATCC 6744) for 1 h at room temperature. Soiled conveyor chips were dipped in poultry rinses contaminated with *Salmonella* or *Listeria* cocktail and incubated at 10 degrees C for 48 h. The polyurethane with mono-polyester fabric conveyor belt and chip exhibited a higher (P<0.05) mean number of attached *Salmonella* serovars (clean: 1.6 to 3.6 cfu/cm2; soiled: 0.8 to 2.4 cfu/cm2) and *L. monocytogenes* (clean: 4.0 to 4.3 cfu/cm2; soiled: 0.3 to 2.1 cfu/cm2) in both clean and soiled conditions. The stainless steel conveyor belt attached a lower (P<0.05) number of *Salmonella* serovars (clean: 0 to 2.6 cfu/cm2; soiled: 0.4 to 1.3 cfu/cm2) and *L. monocytogenes* (clean: 0.4 to 2.9 cfu/cm2; soiled: 0 to 0.7 cfu/cm2) than the polymeric materials, indicating weaker adhesion properties. Plastic conveyor belts exhibited stronger bacterial adhesion compared with stainless steel. The result suggests the importance of selecting the design and finishes of conveyor belt materials that are most resistant to bacterial attachment.

234. Wales, A., M. Breslin, et al. (2007). "A longitudinal study of environmental *Salmonella* contamination in caged and free-range layer flocks."
Avian Pathol **36**(3): 187-197.
http://www.ncbi.nlm.nih.gov/pubmed/17497330
The environmental contamination by *Salmonella* was examined over a 12-month period in 74 commercial layer flocks from eight farms in the UK, which previously had been identified as being contaminated with salmonella. Samples of faeces, dust, litter, egg belt spillage and wildlife vectors were taken, plus swabs of cages, feeders, drinkers, floors, egg belts and boots. Some sampling was performed in each month of the year. Numerous serovars were detected but *Salmonella enterica* serotype Enteritidis was the only persistent serotype found among single-age flocks. There was a significant correlation between qualitative environmental samples and semi-quantitative faeces samples. The level of environmental contamination increased significantly over time. There were significant temperature and seasonal effects upon contamination. Wildlife vectors proved to be sensitive samples for the detection of salmonella. The efficacy of cleaning and disinfection upon residual salmonella contamination, and upon subsequent flock contamination, was highly variable between and within premises. The variability between detected prevalences over time and between flocks indicates a need for regular, sensitive monitoring of flocks for salmonella to permit targeting of control measures aimed at eliminating contamination of the layer environment by salmonella. There is substantial scope for improvement of cleaning and disinfection procedures.

235. Wales, A. D. and R. H. Davies (2011). "A critical review of *Salmonella* Typhimurium infection in laying hens."
Avian Pathol **40**(5): 429-436.
http://www.ncbi.nlm.nih.gov/pubmed/21879803
Salmonella Typhimurium has been reported to contaminate egg production across the world, but where *Salmonella* Enteritidis is endemic it is this latter serovar that dominates egg-borne salmonellosis. However, *Salmonella* Typhimurium is a major food-borne pathogen so it is

important to understand how it can impact the microbiological safety of eggs and what serovar-specific control strategies may be appropriate in the future as control over *Salmonella* Enteritidis continues to improve. To that end, the present review examines the published literature on *Salmonella* Typhimurium in laying hens and eggs, with particular reference to comparative studies examining different serovars. Experimentally *Salmonella* Enteritidis is more often isolated from egg contents and seems to adhere better to reproductive tract mucosa, whilst *Salmonella* Typhimurium appears to provoke a more intense tissue pathology and immune response, and flock infections are more transient. However, it is observed in many cases that the present body of evidence does not identify clear differences between specific behaviours of the serovars Typhimurium and Enteritidis, whether in laying hens, in their eggs, or in the laying environment. It is concluded that further long-term experimental and natural infection studies are needed in order to generate a clearer picture.

236. Wang, H. and M. F. Slavik (1998). "Bacterial penetration into eggs washed with various chemicals and stored at different temperatures and times."
J Food Prot **61**(3): 276-279.
http://www.ncbi.nlm.nih.gov/pubmed/9708295
Eggs were washed with one of three commercial egg-washing chemicals, including a quaternary ammonium compound (QAC, pH 7.5), sodium carbonate (Na2CO3, pH 12), and sodium hypochlorite (NaOCl, 100 ppm, pH 7.5). One hundred fifty intact-shell eggs were washed at 43.3 degrees C with each of three chemicals. A control group was washed with tap water (H2O, pH 7.0). The washed eggs then were inoculated by immersion for 3 min into an aqueous suspension of *Salmonella* Enteritidis at 10(4) colony-forming units/ml and dried for 30 min. The washed and inoculated eggs were stored at 4 degrees C or 23 degrees C, and bacterial penetration was checked at 0-, 7-, 14-, and 21-day intervals. The effects of egg-washing chemicals on microstructural changes of eggshell and postwash inoculation were examined using electron microscopy and conventional culture methods. Fifteen eggs were used in each sample. The results of microbial tests showed that both QAC and sodium hypochlorite treatments reduced bacterial penetration (less than 3.4% and 6.7%, respectively, on day 1 and 16.7% on day 21). The sodium carbonate treatment facilitated bacterial penetration during egg storage (less than 30% on day 1 and 76.7% on day 21). The eggs washed with tap water had a bacterial penetration rate of less than 6.7% on day 1 and 20% on day 21. As the storage intervals increased to 21 days, the bacterial penetration rate increased. Different storage temperatures (4 degrees C and 23 degrees C) did not cause a significant difference in bacterial penetration rates within 21-day interval. The results of electron microscopy showed that QAC and sodium hypochlorite at 100 ppm resulted in microbiologically clean eggs and did not destroy eggshell surfaces, which protected the eggs against future bacterial recontamination. The alkaline sodium carbonate produced visually clean eggs but altered the eggshell surface, which allowed bacterial recontamination.

237. Whiley, H. and K. Ross (2015). "*Salmonella* and eggs: from production to plate."
Int J Environ Res Public Health **12**(3): 2543-2556.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4377917/
Salmonella contamination of eggs and egg shells has been identified as a public health concern worldwide. A recent shift in consumer preferences has impacted on the egg industry, with a push for cage-free egg production methods. There has also been an increased desire from consumers for raw and unprocessed foods, potentially increasing the risk of salmonellosis. In response to these changes, this review explores the current literature regarding *Salmonella* contamination of eggs during the production processing through to food handling protocols. The contamination of eggs with Salmonella during the production process is a complex issue, influenced by many variables including flock size, flock age, stress, feed, vaccination, and cleaning routines. Currently there is no consensus regarding the impact of caged, barn and free range egg production has on *Salmonella* contamination of eggs. The literature regarding the management and control strategies post-collection, during storage, transport and food handling is also reviewed. Pasteurisation and irradiation were identified as the only certain methods for controlling *Salmonella* and are essential for the protection of high risk groups, whereas control of temperature and pH were identified as potential control methods to minimise the risk for foods containing raw eggs; however, further research is required to provide more detailed control protocols and education programs to reduce the risk of salmonellosis from egg consumption.

238. Whiting, R. C. and R. L. Buchanan (1997). "Development of a quantitative risk assessment model for *Salmonella* Enteritidis in pasteurized liquid eggs."
Int J Food Microbiol **36**(2-3): 111-125.
http://www.ncbi.nlm.nih.gov/pubmed/9217100
The performance of hazard analyses and the establishment of critical limits by the food industry are both hampered by the inability to directly relate food processing operations from farm-to-table with their public health impact. Using a 'unit operations' and stochastic simulation approach, data on the frequency of pathogens in raw ingredients, predictive microbiology models for growth and inactivation (thermal and non-thermal), and dose-response models for infectivity were integrated to create a quantitative risk assessment model for a *Salmonella* Enteritidis infection from thermally processed liquid whole eggs made into mayonnaise in the home. The risk assessment indicated pasteurization provides sufficient consumer protection from a high incidence of infected birds and from temperature abuse between the farm and the egg breakers. However scenarios showed how inadequate pasteurization temperatures and/or temperature abuse during storage leads to a hazardous product. This dynamic approach to modeling risk should aid in identification and setting critical control points and assessing the impact of altering food formulations or processes.

239. WHO/FAO, W. H. O. (2002). General principles of food hygiene. Codex Alimentarius.

240. WHO/FAO, W. H. O. (2002). Risk Assessments of *Salmonella* in eggs and broiler chickens, WHO Library Cataloguing-in-Publication Data.
The Members of the Food and Agriculture Organization of the United Nations (FAO) and of the World Health Organization (WHO) have expressed concern regarding the level of safety of food both at the national and the international levels. Increasing foodborne disease incidence over the last decades seems, in many countries, to be related to an increase in disease caused by microorganisms in food. This concern has been voiced in meetings of the Governing Bodies of both Organizations and in the Codex Alimentarius Commission. It is not easy to decide whether the suggested increase is real or an artefact of changes in other areas, such as improved disease surveillance or better detection methods for microorganisms in foods. However, the important issue is whether new tools or revised and improved actions can contribute to our ability to lower the disease burden and provide safer food. Fortunately new tools, which can facilitate actions, seem to be on their way.

241. WHO/FAO, W. H. O. (2007). Code of hygienic practice for eggs and egg products, Codex Alimentarius.
This Code of Hygienic Practice for Eggs and Egg Products takes into consideration, to the extent possible, the differing egg and egg product production systems and processing procedures used by countries. This Code focuses primarily on eggs produced from domesticated chickens. The principles may also be applied to the hygienic practices for egg production from other domesticated egg producing bird species (e.g. duck, quail and goose). Therefore, the code is, of necessity, a flexible one to allow for different systems of control and prevention of contamination of eggs and egg products.

242. Wilhelm, B., A. Rajic, et al. (2011). "The effect of hazard analysis critical control point programs on microbial contamination of carcasses in abattoirs: a systematic review of published data."
Foodborne Pathog Dis **8**(9): 949-960.
http://www.ncbi.nlm.nih.gov/pubmed/21568689
Hazard analysis critical control point (HACCP) programs have been endorsed and implemented globally to enhance food safety. Our objective was to identify, assess, and summarize or synthesize the published research investigating the effect of HACCP programs on microbial prevalence and concentration on food animal carcasses in abattoirs through primary processing. The results of microbial testing pre- and post-HACCP implementation were reported in only 19 studies, mostly investigating beef (n=13 studies) and pork (n=8 studies) carcasses. In 12 of 13 studies measuring aerobic bacterial counts, reductions were reported on beef (7/8 studies), pork (3/3), poultry (1/1), and sheep (1/1). Significant ($p<0.05$) reductions in prevalence of *Salmonella* spp. were reported in studies on pork (2/3 studies) and poultry carcasses (3/3); no significant reductions were reported on beef carcasses (0/8

studies). These trends were confirmed through meta-analysis of these data; however, powerful meta-analysis was precluded because of an overall scarcity of individual studies and significant heterogeneity across studies. Australia reported extensive national data spanning the period from 4 years prior to HACCP implementation to 4 years post-HACCP, indicating reduction in microbial prevalence and concentration on beef carcasses in abattoirs slaughtering beef for export; however, the effect of abattoir changes initiated independent of HACCP could not be excluded. More primary research and access to relevant proprietary data are needed to properly evaluate HACCP program effectiveness using modeling techniques capable of differentiating the effects of HACCP from other concurrent factors.

243. Xiong, R., G. Xie, et al. (1999). "The fate of *Salmonella* Enteritidis PT4 in home-made mayonnaise prepared with citric acid."
Lett Appl Microbiol **28**(1): 36-40.
http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2672.1999.00473.x/epdf
The fate of *Salmonella* Enteritidis PT4 in home-made mayonnaise prepared with citric acid solution (citric acid concentration of Ð4·98% (w/v)) was investigated. It was found that pH of mayonnaise is closely related to the ratio of egg yolk to citric acid, and the inactivation rate of the micro-organisms increases as the ratio decreases and/or incubation temperature increases. To achieve Salm. enteritidis PT4-free home-made mayonnaise prepared with pure lemon juice (citric acid concentration Ð5% (w/v)), it is recommended that the pH should be 3·30 or below, or, in practice, at least 20ml pure lemon juice per fresh egg yolk should be used. For the use of 20–35ml pure lemon juice per egg yolk, the product should be held at 22°C or over for at least 72h and for the use of over 35ml pure lemon juice per egg yolk, for at least 48h before consumption or refrigeration.

5 Plain English Summary

Project Title:	Salmonella Initiative
AECL Project No.	1RD121
Researchers Involved	K A Hewson and R Chia
Organisations Involved	Australian Egg Corporation Limited
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Objectives	To initiate discussion between through-chain stakeholders to determine what constitutes appropriate management of *Salmonella* at each stage through-chain.
Background	A reputational and public health issue for the egg industry is the presence of *Salmonella* spp. (particularly some Typhimurium serotypes), which can cause salmonellosis in humans, throughout the supply chain. The presence and spread of *Salmonella* depends on numerous variables so there is no single effective control measure. Further, as no one business through-chain operates similarly, the identification, level and management of risks will vary, which means that there is not one 'best practice'. Businesses in the supply chain must identify their own 'appropriate' best practice. These issues make the control of *Salmonella* a complex issue subject to a combination of both real and perceived risks.
Research	The AECL *Salmonella* Initiative has engaged with numerous key stakeholders, including state regulators/health departments, industry associations, egg producers, researchers and technical consultants (including veterinarians) to identify and collate information regarding *Salmonella* risk management so that appropriate interventions are able to be implemented through-chain without large impost on any business, yet providing a high level of intervention.
Outcomes	This report should be used to initiate discussion between through-chain stakeholders to determine what constitutes appropriate management of *Salmonella* at each stage through-chain.
Implications	Given the extensive number of stakeholders and the fluid nature of *Salmonella* information this consultation process, and by extension this document, should not remain static. Rather, it should be regularly updated to ensure that any new information that may impact on *Salmonella* risk management anywhere through-chain can be better managed.

Key Words	*Salmonella*; eggs; risk
Publications	*Salmonella* workshops presentation in Nambour and Toowoomba Queensland *Salmonella* presentation to Queensland representative from each council and shire *Salmonella* presentation at the Victoria Department of Human Health and Services

6 Appendices

1. (Thomas, Daughtry et al. 2006 **An Egg: *Salmonella* quantitative risk assessment model**)

2. (Daughtry, Sumner et al. 2005 **National food safety risk profile of eggs and egg products**) + attachments