



## **POULTRY CRC LTD**

### **FINAL REPORT**

Sub-Project No: 3.2.6

SUB-PROJECT LEADER: Dr Kapil Chousalkar

**Sub-Project Title:**  
**Epidemiology of *Salmonella* spp**  
**on free range farms**

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*Epidemiology of Salmonella spp on free range farms*  
*Sub-Project No. 3.2.6*

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## Executive Summary

Eggs produced in Australia are considered medium to low risk food from a food safety viewpoint. The medium risk ranking is mainly because of the food safety impact of pathogens like *Salmonella*. Practically, it would not be possible to completely prevent egg contamination in egg production systems although it is essential to monitor the presence of food borne pathogens present in, or on, eggs collected from layer farms. Longitudinal studies are an appropriate way to address the possible transmission of different *Salmonella* serovars from the environment to the egg, but the requirements of resource, practical difficulties and cooperation by producers over months or years tends to limit the number and scope of such studies. The current project investigated the shedding dynamics of *Salmonella* serovars on commercial free range layer farms. Several *Salmonella* serovars were isolated from the study farms. *S. Typhimurium* phage types 135 and 135a were isolated from two free range farms. All egg internal contents were negative for *Salmonella* spp. The Multiple-Locus Variable number tandem repeat Analysis (MLVA) profile of *S. Typhimurium* isolated from free range flocks sampled in this study and human *Salmonella* cases were similar. Wild birds and foxes appear to play an important role in *S. Typhimurium* ecology and food safety in the free range production system. Free range flocks are exposed to several environmental stressors which can ultimately result in increased *Salmonella* shedding in laying hens. Stress can have an immunosuppressive effect in laying hens which could influence *Salmonella* infection and shedding. In the current study, overall, *Salmonella* shedding was higher in faeces during the summer season. There was no positive correlation between fecal corticosterone metabolites and the level of *Salmonella* shedding. There was no correlation between bird's welfare parameters and the level of *Salmonella* shedding. The outcomes of this project provide important information for the egg industry about the dynamics of *Salmonella* shedding and the possible link between environment/bird/egg transmission of *Salmonella* serovars of public health significance on free range layer farms. *Salmonella* was able to survive in various environmental samples. The level of shedding was higher in summer. The incidence of *Salmonella* spp in the Australian egg industry needs to be monitored regularly.

## Introduction

In Australia, there have been increases in the number of isolations of *Salmonella* Typhimurium from cases of human salmonellosis, acquired from poultry egg products (Moffatt et al., 2016). Control of *Salmonella* on farm and in the egg supply chain still remains a challenge facing the egg industry. The industry therefore needs to be vigilant in monitoring the presence of food borne pathogens such as *Salmonella* on egg shells or in eggs to enable informed management decisions to be made. Although there is the potential for vertical transmission of *Salmonella* (as has been shown to occur for *Salmonella* Enteritidis), it is generally accepted that horizontal transmission is the most likely source of contamination of shell eggs with most bacteria. Longitudinal studies are an appropriate way to address the possible transmission of different *Salmonella* serotype from environment to egg, but the requirements of resource, practical difficulties and cooperation by producers over months or years tends to limit the number and scope of such studies.

In Australia, there are few scientific reports that have examined the levels of *Salmonella* in the free range production system and the actual internal or external egg contamination or quantitative dynamics of *Salmonella* shedding. Currently, longitudinal studies are under way to address the possible transmission of different *Salmonella* serotype from environment/bird to egg on cage farms (Poultry CRC project 3.2.3). During our longitudinal study on cage layer farms, the most commonly isolated *Salmonella* serovars were, *Salmonella* Infantis, subspecies 1 serovar 4, 12: d, Agona, Orion, Orion var 15+, 34+, Typhimurium (PT9) and *Oranienburg*. Some of these serotype, notably *Salmonella* Infantis, Oranienburg and Typhimurium (PT9) have also been isolated from egg shell washes. It was also observed anecdotally that certain stressors (such as transport or moulting) in controlled environment could trigger *Salmonella* shedding in a flock.

Birds raised in free range production systems are potentially exposed to more environmental stressors than caged birds, including social stress and aggression, predation or thermal

challenges. The free range production system is becoming a major source of egg production in Australia. Epidemiological studies are helpful to understand the sources and chains of transmission to plan and apply prevention measures.

In the Australian free range egg production system, all birds are required to have access to the range for a minimum of 8 hours per day once they are reasonably feathered i.e. by onset of lay. The only exception to this is during extreme weather conditions (e.g. exceptionally hot or cold weather, high humidity, very strong winds or heavy rain) or under veterinary advice (e.g. due to a disease outbreak). Egg producers also have to meet the egg production standards designed by supermarkets. Ranging in extreme weather conditions could be stressful for the birds and may increase *Salmonella* shedding. The proposed study investigated the possible transmission of different *Salmonella* serotype from environment/bird to egg on free range farms. The effects of stressors on the level of *Salmonella* shedding were investigated.

## Objectives

- The current study will assist in determining the link between environment/bird/egg transmission of *Salmonella* spp in free range egg layer farm.
- To study the effect of stage of lay on *Salmonella* shedding
- To study the possible relationship between level of egg contamination and *Salmonella* load in the poultry shed environment.
- Investigate the effects of environmental stressors on the level of *Salmonella* shedding in free range flocks.
- To develop practical guidelines/recommendations for management of stress to reduce *Salmonella* shedding in the flock.

## Methodology, Results and Discussion

The methodology, results and discussions of this work have been presented in the two scientific manuscripts arising from this project. The manuscript details are provided below and are attached to this Report.

### Manuscript 1

Chousalkar, K., Gole, V.C., Caraguel, C. and Rault, J-L. (2016). Chasing *Salmonella* Typhimurium in free range egg production system. *Veterinary Microbiology*, 192, 67–72

### Manuscript 2

Gole, V.C., Woodhouse, R., Carguel, C., Moyle, T., Rault J-L., Sexton, M. and Chousalkar K. (2016). Dynamics of *Salmonella* shedding and behaviour of hens in free range production systems. Prepared for *Scientific Reports*.

## Implications

The current project conducted longitudinal and point in-time surveys of *Salmonella* carriage and environmental contamination on commercial free range layer farms. The data provides critical information for the egg industry about dynamics of *Salmonella* shedding and the possible link between environment/bird/egg transmissions of *Salmonella* serovar of public health significance on commercial free range layer farms. *Salmonella* isolates were able to survive in faeces, egg belt and dust and result in egg shell contamination. The level of *Salmonella* shedding in feces was numerically higher in summer across the

entire study which involved five farms. While not a statistically significant difference, this higher shedding level indicates that temperature related stress may be associated with *Salmonella* shedding. However it is important to note that fecal corticosterone metabolites (FCM) were not higher in summer. During this study, the internal content of eggs laid by *S. Typhimurium* positive hens were negative for *Salmonella*. *Salmonella* Typhimurium strains responsible for human food poisoning cases exhibited similar MLVA pattern to the strains isolated from flocks C and D. The level of *Salmonella* shedding did not influence behavior of birds in general. There was no positive correlation between behavior parameters and *Salmonella* shedding and or prevalence. There was no positive correlation between the level of corticosterone in fecal samples and *Salmonella* shedding. *S. Mbandaka* was the most prevalent serovar during this study followed by *S. Typhimurium*. Almost all serovars possessed genes associated with virulence. Free range flocks are challenged by environmental stressors and it is difficult to implement biosecurity protocols to control *Salmonella*. Various on-farm interventions can be adopted to control the level of *Salmonella* shedding but strict hygiene measures and sanitation programs in food processing facilities and the food chain in general are required.

## Recommendations

- The investigators recommend that the Australian Egg Industry continues to support research, development and extension in the area of food safety, with *Salmonella* and certain serovars such as *S. Typhimurium* being the main target.
- Extension activities arising from the outcomes of this sub-project could include seminars and workshops for the egg producers and health department officials. Such extension activities have already been conducted by the investigators as part of national egg and *Salmonella* workshop for regulators and egg producers.
- Since stressful events such as exposure to heat could influence the *Salmonella* shedding in laying hens, efforts should be directed to minimising the stress level in flock.
- More controlled experiments are required to understand the effects of stress on the level of *Salmonella* shedding.
- After depopulation, a thorough cleaning of layer shed equipment (slats, egg belt, egg belt brushes, feeders, floor, nesting boxes, nest pads) and areas such as ventilation fans and cage tops is essential.
- It is also essential to study the shedding of *Salmonella* in pullets during rearing as the current study investigated shedding during lay or week 24 onwards.
- A nationwide epidemiological survey of *Salmonella* could be conducted in future. The current study was conducted on five layer flocks in two states
- Studies could be conducted to investigate the link between increase in *Salmonella* shedding on farm and high human *Salmonella* notifications in summer. The behaviour of *Salmonella* in egg supply chain or in commercial kitchen environment is a missing link.
- Wild birds and foxes appear to play an important role in *S. Typhimurium* ecology and food safety in free range production systems. Effective measures are required to minimize their contact with poultry and eggs.

## **Acknowledgements**

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## **References**

Moffatt, C.R., Musto, J., Pingault, N., Miller, M., Stafford, R., Gregory, J., Polkinghorne, B.G., Kirk, M.D., 2016. *Salmonella Typhimurium* and Outbreaks of Egg-Associated Disease in Australia, 2001 to 2011. *Foodborne Pathog Dis* 13, 379-385.

## POULTRY CRC

### Plain English Compendium Summary

<b>Sub-Project Title:</b>	<b>Epidemiology of <i>Salmonella</i> spp on free range farms</b>
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<b>Sub-Project Overview</b>	The current project investigated shedding dynamics of <i>Salmonella</i> serovars on commercial free range layer farms. Several <i>Salmonella</i> serovars were isolated from farms. S. Typhimurium phage types 135 and 135a were isolated from two free range farms. All egg internal contents were negative for <i>Salmonella</i> spp. The MLVA profile of S. Typhimurium isolated from free range flocks sampled in this study and human <i>Salmonella</i> cases were similar. <i>Salmonella</i> shedding in faeces was higher in faeces during the summer season. There was no positive correlation between fecal corticosterone metabolites and the level of <i>Salmonella</i> shedding. There was no correlation between bird's welfare parameters and level of <i>Salmonella</i> shedding and or prevalence.
<b>Background</b>	It is widely recognised that <i>Salmonella</i> spp are a potential threat to the chicken meat and egg industry. Although egg producers are diligent in fulfilling standards for the production of safe food, the egg industry in Australia is often implicated in some outbreaks of food poisoning. The industry therefore needs to be vigilant in monitoring the presence of food borne pathogens such as <i>Salmonella</i> on farm to enable informed management decisions to be made.
<b>Research</b>	
<b>Sub-Project Progress</b>	The work has been completed. All milestones and objectives have now been achieved.
<b>Implications</b>	The data provides important information for the egg industry about dynamics of <i>Salmonella</i> shedding and the possible link between environment/bird/egg transmission of <i>Salmonella</i> serovars of public health significance on free range layer farms. <i>Salmonella</i> was able to survive in various environmental samples. The level of shedding was high in summer. The incidence of <i>Salmonella</i> spp needs to be monitored regularly in the Australian egg industry.
<b>Publications</b>	1 manuscript published, 1 manuscript to be submitted Delivered presentations at 13 different workshops conducted on Egg and <i>Salmonella</i> .