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Kapil K. Chousalkar, Margaret Sexton, Andrea McWhorter, Kylie Hewson, Glen Martin, Craig Shadbolt & Paul Goldsmith

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***Salmonella* Typhimurium in the Australian egg industry: Multidisciplinary approach to addressing the public health challenge and future directions**

Kapil K. Chousalkar¹, Margaret Sexton², Andrea McWhorter¹, Kylie Hewson³, Glen Martin⁴, Craig Shadbolt⁵, Paul Goldsmith⁶

¹The University of Adelaide, SA, Australia.

²Primary Industries and Regions, SA, Australia.

³Australian Egg Corporation Limited,

⁴Food and Controlled Drugs Branch, SA Health,

⁵NSW Food Authority

⁶Food Safety Unit, Victoria

Corresponding Author: Dr. K. K. Chousalkar, School of Animal and Veterinary Science, The University of Adelaide, Roseworthy, 5371, SA, Australia, Telephone: +61 8 8313 1502, Facsimile: + 61 8 8303 7356, Email: kapil.chousalkar@adelaide.edu.au

Key words

Eggs, *Salmonella* Typhimurium, laying hens, food handling, communication

Abstract

In Australia, numerous egg related human *Salmonella* Typhimurium outbreaks have prompted significant interest amongst public health authorities and the egg industry to jointly address this human health concern. Nationwide workshops on *Salmonella* and eggs were conducted in

Australia for egg producers and regulatory authorities. State and National regulators representing Primary Production, Communicable Disease Control, Public Health and Food Safety and Food Standards Australia and New Zealand. All attendees participated in discussions aimed at evaluating current evidence based information, issues related to quality egg production and how to ensure safe eggs in the supply chain, identifying research gaps and practical recommendations. The perceptions from egg producers and regulatory authorities from various states were recorded during the workshops. We presented the issues discussed during the workshops including *Salmonella* in the farm environment, *Salmonella* penetration across egg shell, virulence in humans, food/egg handling in the supply chain and intervention strategies. We also discussed the perceptions from egg producers and regulators. Recommendations placed emphasis on future research needs, communication between industry and regulatory authorities and education of food handlers. Communication between regulators and industry is pivotal to control egg borne *S. Typhimurium* outbreaks and collaborative efforts are required to design effective and appropriate control strategies.

Introduction

It is widely recognised that *Salmonella* are a potential threat to the chicken meat and egg industries due to the negative implications for human health. Particularly, consumption of under cooked egg products are often implicated in *Salmonella* outbreaks (Gast, 2007; OzFoonet, 2012). In Europe and the USA, *Salmonella* Enteritidis is a major concern because of its significant negative implications for human foodborne illness. Although *Salmonella* Enteritidis is common as a cause of human gastroenteritis in Australia, it has not been linked to the Australia egg industry, however, other *Salmonella* serovars such *Salmonella* Typhimurium (*S. Typhimurium*) are still of interest. Egg products associated *S. Typhimurium* outbreaks have been frequently reported in Australia (OzFoonet, 2010, 2012; Stephens, Coleman, & Shaw, 2008; Stephens, Sault, Firestone, Lightfoot, & Bell, 2007). Numerous egg related human *S. Typhimurium* outbreaks have prompted significant interest amongst the general public, public health authorities and industry. The 2015 Nationwide workshops on *Salmonella* and eggs were conducted in Australia for egg producers and regulatory authorities. More than 80 commercial egg producers and independent veterinary consultants attended the workshop. More than 75 individuals attended sister workshops conducted for regulators. State and National regulators representing Primary Production, Communicable Disease Control (including OzFoodNet and Public Health Registrars) , Public Health and Food Safety and , Food Standards Australia and New Zealand. The survey was conducted during the workshop to elicit perceptions of workshop attendees with regard to egg related salmonellosis, via electronic audience polling. The participation in this survey was voluntary and response was anonymous. The response from audience was based on their knowledge and breadth of experience about egg related salmonellosis.

The major objectives of the workshop were:

- “ To initiate discussion between egg industry and regulatory authorities, discuss on-farm risk factors and intervention strategies on and off the egg farm.
- “ To obtain information from participants about their understanding of issues to help the egg industry funding body to focus on priority R&D areas.
- “ To provide evidence based information to regulators and egg producers, review current practises on farm and also in supply chain.
- “ To improve general understanding among stakeholders on farm issues and supply chain issues that relate to food safety and to gain insight into their priorities and perspectives.

In this report we present the current evidence based information and workshop discussions. We also discussed the survey results collected from the workshop participants. The proposed article also involves, discussion around perspectives of regulators and farmers against the evidence based information, limitations regarding current regulations/policies, limitation of disease notification systems, future research needs and future possible review of some regulatory practises. Recommendation on future research needs and communication between industry and regulatory authorities are also discussed.

***S. Typhimurium* in the egg industry and trace back investigation**

Production of visually clean eggs, free from dirt and faecal contamination, is the primary concern in the supply of table eggs, although, visually clean eggs do not necessarily guarantee food safety. Surveys collected during the workshops indicated that for majority of egg producers rated that, egg safety is an important aspect of egg production (Supplementary Fig 1a). Most of these *S. Typhimurium* have been frequently isolated from the egg farm environment (McWhorter &

Chousalkar, 2015; McWhorter, Davos, & Chousalkar, 2015). Egg related *Salmonella* outbreaks have been reported in most parts of the world (Martelli & Davies, 2012).

The national reporting system of human salmonellosis and role of respective regulatory authority is described in Figure 2. Laboratory confirmed infections of *Salmonella* are reported to the Communicable Disease Control (CDC) sections within State and Territory Health Departments. Each jurisdiction in Australia has different procedures for follow up of *Salmonella*. The CDC assesses each *Salmonella* notification to determine if there is a cluster or an outbreak. When public health follow up is warranted, cases are interviewed to ascertain exposure to potential sources of *Salmonella*. Often the time of interview is 3-4 weeks after the exposure period, which can make it challenging to establish the exact source of the infection.

During the workshops, a large proportion of regulators believed that Multiple-Locus Variable number tandem repeat Analysis (MLVA) testing has improved the trace back investigations during the *Salmonella* outbreaks (Supplementary Fig 1b). Frequently, multiple MLVA types of *S. Typhimurium* are identified during human outbreaks (Octavia et al., 2015). MLVA and whole genome sequencing techniques are helpful for discrimination of definitive types within the same serovar but were unable to detect the virulence of bacteria.

Commercial egg farms in Australia and grading plants are audited by food safety authorities by the respective state authorities and/or audited against the voluntary egg industry QA program managed by the Australian Egg Corporation Ltd, and in some cases individual customer audits are also required. Small scale backyard poultry owners must comply with the same standards as larger producers however regulatory surveillance is restricted to investigation if they are implicated in food borne illness reports to reduce regulatory burden. It was considered that such

businesses if not managed properly could be a source for egg related *Salmonella* outbreaks. From the survey conducted during the workshop, a large proportion of regulators believed cracked and dirty eggs and the kitchen environment were the major risks for *Salmonella* proliferation (Supplementary Fig 1c) and interestingly regulators from all states voted transport and egg grading floor to be of lower risk. There was a small segment in the regulatory authority who believed that *S. Enteritidis* is prevalent in the commercial egg industry (Supplementary Fig 1d). It is important to monitor the presence of *S. Enteritidis* through regular surveillance.

During the workshop, it was discussed that the best before date on Australian eggs is based on egg quality factors rather than microbiological quality of the product. Given the possibility of *S. Typhimurium* survival on the egg shell surface of washed and unwashed eggs for up to three weeks post infection (Gole, Chousalkar, et al., 2014), the guidelines regarding the Australian table egg best before date needs to be reviewed. Also, research is required to determine the ability of *S. Typhimurium* survival on the egg shell surface beyond three weeks of infection.

Prevalence and Epidemiology of *Salmonella* on egg layer farms

According to Australian food safety authorities, *Salmonella* contamination of eggs and egg products is a major public health issue; however there is conflicting data amongst egg based prevalence studies. In Australia, surveys to date have been conducted using large or small number of eggs from the layer farms. During 1986, 360 eggs from wholesale and retail markets in Cairns and Townsville were all negative for *Salmonella*. A 1989 survey involving 199 eggs was also completely negative for *Salmonella* (Douglas, 2004). *Salmonella* spp. were not isolated from the external surface of 10,000 eggs or the internal contents of 20,000 commercial eggs sampled (Daughtry et al., 2005). A study conducted on isolation of *Salmonella* from egg shell

wash, egg shell pores and internal contents using a relatively small number of samples revealed that the egg shell and egg internal contents were negative (Chousalkar, Flynn, Sutherland, Roberts, & Cheetham, 2010). However in a later study performed in 2012, *Salmonella* Infantis was isolated from egg shell wash (Chousalkar & Roberts, 2012). It was reported that implied prevalence of *Salmonella* on retail egg on shelf was 0.30 % (Fearnley, Raupach, Lagala, & Cameron, 2011). It is important to note that *S. Typhimurium* was not isolated from eggs during any of the egg based survey however eggs and raw egg products have still being associated with *Salmonella* outbreaks. Egg based surveys are important but laborious due to the large number of eggs required for *Salmonella* testing. In Australia, 392 million dozen eggs are produced annually (AECL, 2013) hence reliance on survey of small number of eggs may not be an accurate or true reflection of *Salmonella* prevalence.

Longitudinal studies are an appropriate way to address the possible transmission of different *Salmonella* serotypes from the environment to the egg. However the resources required for the research, practical difficulties and obtaining cooperation from producers (over months or years) limits the number and scope of such studies. There are few reports that have examined the levels of *Salmonella* in laying houses and hens in lay over an extended period of time (Davison, Benson, Henzler, & Eckroade, 1999); (Davies & Breslin, 2003); (Kinde et al., 2005) and up to a period of 12 months (Wales, Berslin, Carter, Sayers, & Davis, 2007). According to a recent longitudinal investigation (Gole, Torok, Sexton, Caraguel, & Chousalkar, 2014), egg internal contents of *S. Typhimurium* shedding birds tested negative but the level of egg shell contamination was up to 6 log CFU per egg.

A large proportion of regulators believed that egg internal contents of freshly laid eggs are not contaminated (Supplementary Fig 1e). There is little information on nationwide *Salmonella* prevalence on egg farms in Australia. One of the challenges in establishing such prevalence is that the shedding of *S. Typhimurium* could be variable and depends upon the stress level in the flock, stocking density, season, number of samples tested and type of samples tested (Van Hoorebeke, Van Immerseel, Haesebrouck, Ducatelle, & Dewulf, 2011). There was a difference in perception amongst regulators that free range farms were the source of major contamination (Supplementary Fig 1f). There has been further debate on the effects of production systems (cage vs. free range egg production) on the level of *Salmonella* contamination (Van Hoorebeke et al., 2011). The level of *Salmonella* contamination of farms could be highly variable across different flocks and farms hence the level of egg contamination is largely attributed to the individual flock management and or farm management. Moreover, farm, flock size, stocking density, level of stress, carry over infection from pests, hygiene measures also play a significant role (Van Hoorebeke et al., 2011). The survey results from egg producers revealed that there was variation in perception regarding the *Salmonella* infection status of the flock and or farm (Supplementary Fig 1g). There are several possible input sources where *Salmonella* could be introduced in the flock hence further research is required to investigate the longitudinal epidemiology of *S. Typhimurium* from day old to the end of commercial life (75-80 weeks) in laying flocks on both caged and free range farms. In some states, regular samples are collected by the egg farmer (operator), and / or with annual routine (official) samples being collected by the state food safety authority to verify status of the farm. However, egg sampling is not uniformly practised across the country predominately due to the cost of sampling, no (or limited) requirements to sample

and minimal understanding of how to sample a farm for *Salmonella* number, type and source of samples collected from farms are often variable.

Effects of egg quality and *Salmonella* penetration

Anatomically, hens have a common opening of the intestinal, urinary and reproductive tracts which could contribute to external egg shell contamination as the egg passes through this region. Egg quality can play a major role in trans shell penetration of *Salmonella* spp., which could be affected by serovars (De Reu et al., 2006); (Gast, 2007; Gast, Guraya, Guard-Bouldin, & Holt, 2007), temperature difference between egg and external contaminant (Miyamoto et al., 1998), load of bacteria (Miyamoto et al., 1998), egg shell quality (Gole, Chousalkar, et al., 2014), cuticle deposition (Gole, Roberts, et al., 2014), pH of contaminating medium (Sauter, Petersen, Parkinson, & Steele, 1979), relative humidity and moisture (Gast, Holt, & Guraya, 2006), egg shell porosity (De Reu et al., 2006), microcracks (Jones, Lawrence, Yoon, & Heitschmidt, 2011), and general handling in the supply chain. *S. Typhimurium* serovars are able to survive on the egg shell surface of washed and unwashed eggs although the survival ability of *Salmonella* serovars is variable (Gole, Roberts, et al., 2014).

Although an egg has some natural antibacterial defence mechanisms such as cuticle on the shell and antibacterial properties of albumen, these defence barriers could be influenced by some unavoidable practises. At oviposition, while the cuticle is hardening, the egg undergoes a series of temperature changes ($\sim 40^{\circ}\text{C}$ of hens normal body temperature to shed temperature at $\sim 23^{\circ}\text{C}$). This difference in temperature gradient causes negative pressure inside an egg which could then potentiate the transshell penetration of *Salmonella* present on the egg shell or any contaminated surface in the shed. *S. Typhimurium* has the ability to survive in egg white (Gole, Chousalkar, et

al., 2014), however bacteria was not able to multiply in egg white. The presence of yolk can favour the replication of *Salmonella* spp (Gast & Holt, 2001). During the workshop, both egg industry and regulators agreed that presence of egg yolk (due to broken eggs) during transit through the supply chain could amplify the food safety risk.

Although a large proportion of regulators believed that egg internal contents of freshly laid eggs are not contaminated with *Salmonella* spp, there was variation in opinion about the level of *Salmonella* contamination in egg shell membrane and pores (Supplementary Fig 1h). Oiling of eggs after washing is practised in the egg industry, although it is not clear how widespread the practise is in Australia. Oiling is believed to restrict the bacterial movement and help seal the egg shell pores after washing (Waimaleongora-Ek, Garcia, No, Prinyawiwatkul, & Ingram, 2009). During the discussion, the majority of egg producers suggested that further research is required to investigate the effects of oiling on *S. Typhimurium* penetration and survival.

Intervention strategies to control *Salmonella*

Reducing the environmental load of *Salmonella* in the layer shed, by adopting good management practices (such as regular cleaning of sheds), could reduce the incidence of egg contamination. Various methods have been used to control *Salmonella* in layer flocks (Gali et al., 2013). In this manuscript, we mainly focused on egg washing, vaccination and use of organic acids because these practises are widely used in the industry, both nationally and internationally.

Egg washing with sanitizers is one of the most common methods of reducing eggshell contamination. This technique is adopted in many countries such as Australia, Japan and USA (Hutchison et al., 2004). However, some studies suggested that chemicals used in egg washing have the potential to alter the eggshell surface and also can damage the cuticle layer (Gole,

Roberts, et al., 2014). The majority of eggs produced in Australia would be subjected to some form of egg washing as egg washing practises in the industry can be highly variable (Supplementary Fig 1i). Most commercial egg washing machines have an egg contact wash time of around 30 seconds or less. During the egg washing process, a number of factors such as temperature of water, egg temperature (external and internal), efficacy of cleaning agents, sanitizers, functionality of egg washing equipment (egg rollers and brushes, spray nozzles), water quality, consistent supply of fresh chemical in egg washing machines, reuse of washing solutions, functionality of dosing pumps and overall cleanliness of an egg washer are critical aspects (Hutchison et al., 2004). If all above factors are not managed properly during the egg washing process, the egg could further be exposed to high levels of bacterial contamination. The survey results from the egg producers workshop highlighted the variability of egg washing across the Australian egg industry (Supplementary Fig.1j). The survey also highlighted variability within and in between producers from different states. Also, there was variability in several other practises such as egg equipment cleaning, egg filler usage, egg filler and floor egg treatment (Supplementary Figures 1k, 1l, 1m, 1n).

Vaccination to prevent or reduce *Salmonella* infection in poultry has been accepted worldwide and vaccination of pullets is one of the practical measures to reduce *Salmonella* shedding (Desin, Koster, & Potter, 2013). The Vaxsafe® ST (Bioproperties Pty Ltd, Australia) is an *aroA* deletion mutant vaccine (Bachtiar et al., 2003) and is the only live attenuated vaccine available for use in Australia. This vaccine is registered to use for spray and drinking water applications and has been shown to reduce *Salmonella* shedding in broilers (Groves, Sharpe, & Cox, 2015). The long

term efficacy of the vaccine in commercial layer flocks that are actively shedding *Salmonella* in field conditions remains unclear.

A number of different organic acids are included as dietary supplements in the feed and drinking water of poultry to reduce the presence of pathogenic bacteria such as *Salmonella*. Short Chain Fatty Acids can affect the invasion of *Salmonella*, altering these concentrations in the caecum by changing feed composition may prove to be an efficient way of controlling the pathogen (Van Immerseel F et al., 2006). Further studies are essential to establish guidelines for strategic use of dietary organic acid in feed to reduce *Salmonella* colonization/ shedding in layers.

The perception regarding on farm *Salmonella* intervention strategies were variable amongst regulators and egg producers (Supplementary Figures 1o, 1p). There is sufficient literature regarding the *Salmonella* intervention strategies, however at the field level, it is yet to be determined whether implementation of single or multiple intervention strategies could sufficiently reduce the load of *S. Typhimurium* on farm to produce a significant reduction on eggs.

Salmonella Growth in food and its virulence

In Australia, few studies have been conducted to investigate the growth kinetics of *S. Typhimurium* in a variety of egg-based food products at different storage temperatures (Comar, 2012). Although there has been a wide discussion across industry on implementation of a range of on- farm risk mitigation strategies, the risks associated with the food service sector cannot be ignored. *In vitro* work on *Salmonella* virulence characterisation has demonstrated that the post-enrichment, invasive capacity of *S. Typhimurium* increased ten-fold (McWhorter et al., 2015). Therefore, if there is any stage during food preparation or storage that encourages the growth or

enrichment of *Salmonella* within the food item, the risk of potential food poisoning increases. It has been reported in the literature that some specific virulent genes from specific pathogenicity Island 1 (SPI 1) of *Salmonella* were highly expressed after enrichment (Patterson, Borewicz, Johnson, Xu, & Isaacson, 2012). Our recent comparative analysis of whole genome sequence of virulent and non-virulent non typhoidal *Salmonella* serovars indicated that there may be defects in genes of SPI 1, 2, 3, 4 & 5 responsible for variation in intestinal epithelial invasion and replication within the cell and macrophages (McWhorter & Chousalkar, 2015) .

It was agreed during the regulators workshop that further research work is required to understand whether *Salmonella* enriched in food is more virulent (Supplementary Fig 1q). *Salmonella* spp has the capacity to prolong survival in raw egg-based food products even if the products are acidified. Although acidic conditions are not favourable for multiplication of bacteria, other enrichment conditions such as other food ingredients, diet, health (gut health) and status of an individual could further amplify the resulting *Salmonella* spp which could ultimately cause infection.

Education and communication of stakeholders

To control egg related Salmonellosis in humans, the education and communication could not be only limited to egg producers. Several stakeholders involved in egg supply chain are people involved in egg handling (on and off-farm), preparation of commercial egg-based food products, retailers, outbreak investigators, policy makers and more importantly, consumers. Irrespective of egg source (organic, free range or cage), given that eggs are a non-sterile raw animal product, awareness about proper handling in a commercial kitchen environment and general public is essential. Cultural diversity within respective countries has contributed to a far wider selection of

food, incorporating a greater range of raw foods of animal origin into our diet. The efficacy of messages such as 'cook eggs thoroughly' or 'wash your hands' will depend both on the ability to change consumer behaviour as well as identification of where and how the food safety risk can best be mitigated (Luber, 2009). It is also important to note that the food handling behaviour could be influenced by sex, income status and age of an individual (Patil, Cates, & Morales, 2005).

Both regulators and egg industry personnel were of the opinion that more efforts are required at farm level and also in the supply chain. When asked about whether the egg industry is doing enough to control *Salmonella* on farm, the majority of the regulators were negative or uncertain about the industry efforts (Supplementary Fig 1r). Interestingly the majority of egg producers were also uncertain about industry efforts (Supplementary Fig 1s). The majority of egg producers and regulators across the nation agreed that food handling practises in Australian food service outlets are not sufficient to control *Salmonella* growth (Supplementary Figures 1t, 1u).

The nationwide workshops on *Salmonella* and eggs were seen as a first step towards improvement of communication between industry and regulatory authorities. The response from both egg producers and regulators indicated that the current workshops were highly successful and majority of attendees would attend such workshops if conducted in the future (Supplementary Figures 1v, 1w). This highlights the importance of communication between industry and regulatory bodies and that regular communication is essential for controlling food borne human illness. It is important to note that there was variation in the survey results between states. This could be attributed to different views and or opinion of workshop attendees based on their knowledge and breadth of experience.

In conclusion, the issues debated and discussed during the workshop were helpful to obtain information from regulators and producers about their understanding of issues in order to help the egg industry funding body (Australian Egg Corporation Limited) to focus on priority R&D areas in food safety. The debate during the workshops improved general understanding among stakeholders on farm issues and supply chain issues that relate to food safety. The discussions, debates and findings of the nationwide workshops have initiated discussion between egg industry and regulatory authorities, which is initial step towards improving the communication between stakeholders.

Conflict of interest

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article. Dr. Kylie Hewson was employed by Australian Egg Corporation Limited. The remaining authors declare no conflicting interests with respect to their authorship or the publication of this article.

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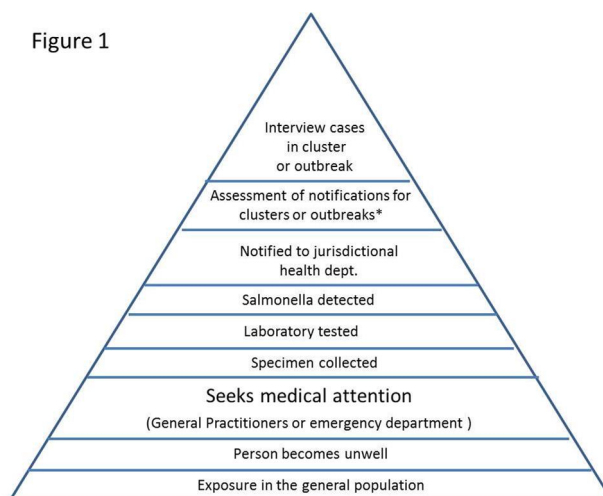
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Figure 1



Current Salmonella infection notification process in Australia

* Individual jurisdictions interview cases depending on the number of notifications and available resources

Figure 1: Current Salmonella infection notification process in Australia. * Individual jurisdictions interview cases depending on the number of notifications and available resources