AUSTRALIAN EGG CORPORATION LIMITED

Report on workshops for development of *Salmonella* control strategies in egg industry

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Foreword

Nationwide workshops were conducted during 2015 on *Salmonella* and eggs, to present scientific and evidence-based findings conducted by the industry. These findings were presented to major commercial egg producers and regulatory authorities in Australia. This communication activity was conducted for the first time in the country with the rationale of initiating the discussions and bringing both industry and regulatory authorities onto the same platform. During the workshop, information was collected regarding the perceptions and understanding of the workshop audience (egg farmers and regulators) on egg-related Salmonellosis.

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.

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Abbreviations

AECL	Australian Egg Corporation Limited
CDC	Communicable disease control
CFU	Colony-forming unit
FSANZ	Food Standards Australia and New Zealand
MLVA	Multiple-Locus Variable number tandem repeat Analysis
NSW	New South Wales
QA	Quality assurance
R&D	Research and development
SA	South Australia
SPI 1	Specific Pathogenicity Island 1
S. Enteritidis	*Salmonella* Enteritidis
S. Typhimurium	*Salmonella* Typhimurium
WA	Western Australia

Executive Summary

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 during 2015 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 (FSANZ) were in attendance. 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. The issues discussed during the workshops included *Salmonella* in the farm environment, *Salmonella* penetration across egg shell, virulence in humans, food/egg handling in the supply chain and intervention strategies.

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.

1 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 (*S.* Enteritidis) is a major concern because of its significant negative implications for human foodborne illness. Although *S.* 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 with *S.* Typhimurium outbreaks have been frequently reported in Australia (OzFoonet, 2010, 2012; Stephens *et al.,* 2008; Stephens *et al.,* 2007). Numerous egg related human *S.* Typhimurium outbreaks have prompted significant interest amongst the general public, public health authorities and industry.

In 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, including State and National regulators representing Primary Production, Communicable Disease Control (including OzFoodNet and Public Health Registrars), Public Health and FSANZ. A 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;
- Obtain information from participants about their understanding of issues to help the egg industry funding body to focus on priority research and development (R&D) areas;
- Provide evidence based information to regulators and egg producers, review current practises on-farm and also in supply chain; and
- Improve general understanding among stakeholders on the farm issues and supply chain issues that relate to food safety and to gain insight into their priorities and perspectives.

This report presents the current evidence-based information, workshop discussions and 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.

2 General commentary on the workshop

2.1 *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 the majority of egg producers rated egg safety as an important aspect of egg production (**Appendix Figure 6-1**). 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 2-1**.

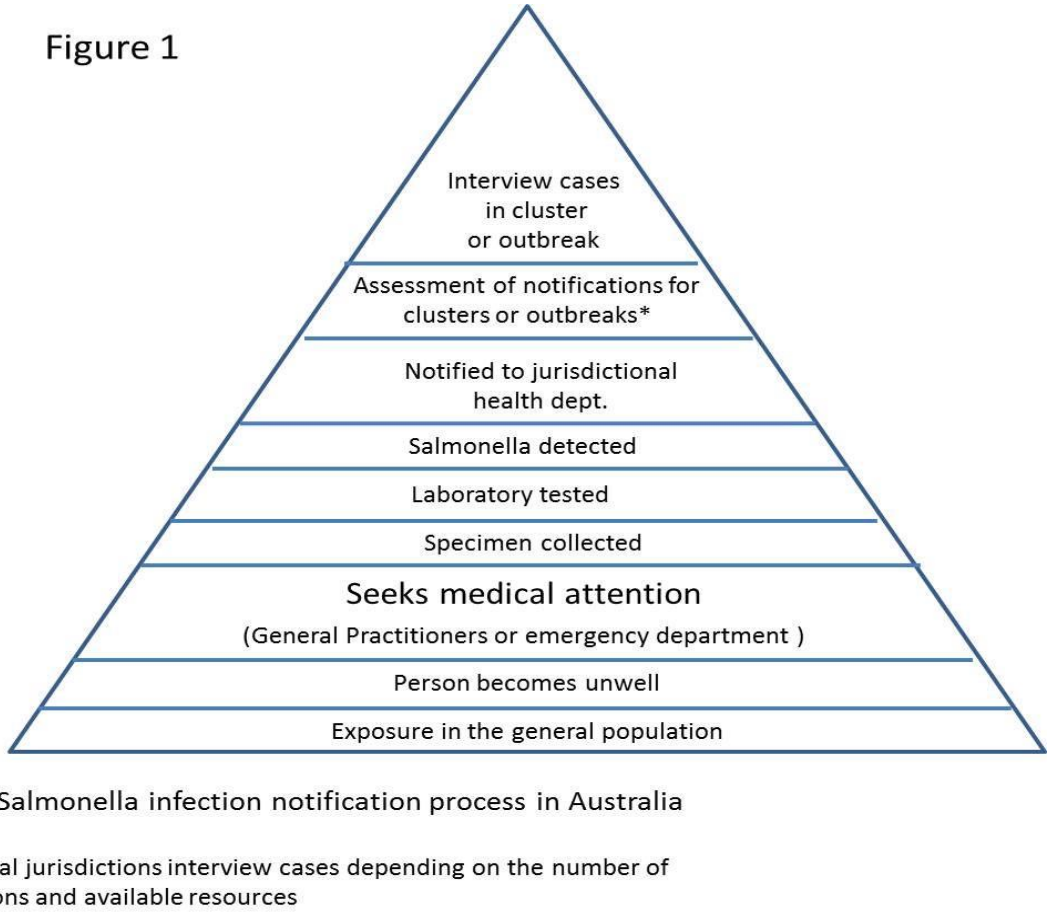

Figure 2-1: Current Salmonella infection notification process in Australia.

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 (**Appendix Figure 6-2**). 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 and grading plants in Australia are audited by food safety authorities, the respective State authorities and/or audited against the voluntary egg industry quality assurance (QA) program managed by AECL, 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, to reduce regulatory burden, surveillance is restricted to investigation if they are implicated in food borne illness reports. 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 (**Appendix Figure 6-3**) 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 that believed that *S.* Enteritidis is prevalent in the commercial egg industry (**Appendix Figure 6-4**). 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.

2.2 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 *et al.*, 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 *et al.*, 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 *et al.*, 1999; Davies & Breslin, 2003; Kinde *et al.*, 2005) and up to a period of 12 months (Wales *et al.*, 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 the internal contents of freshly laid eggs are not contaminated (**Appendix Figure 6-5**). 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 *et al.*, 2011). There was a difference in perception amongst regulators that free range farms were the source of major contamination (**Appendix Figure 6-6**). 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 (**Appendix Figure 6-7**). 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.

2.3 Effects of egg quality and Salmonella *penetration*

Anatomically, hens have a common opening of the intestinal, urinary and reproductive tracts that 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 *et al.* 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 *et al.*, 1979), relative humidity and moisture (Gast *et al.*, 2006), egg shell porosity (De Reu *et al.*, 2006), micro cracks (Jones *et al.*, 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 (~400C of hens normal body temperature to shed temperature at ~230C). This difference in temperature gradient causes negative pressure inside an egg, which could then potentiate the transhell 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 the 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 (**Appendix Figure 6-8**). 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 *et al.*, 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.

2.4 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 (Galiş *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 (**Appendix Figure 6-9**). 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 (**Appendix Figure 6-10**). 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 (**Appendix Figures 6-11, 6-12, 6-13, 6-14**).

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 *et al.*, 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 *et al.*, 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 (**Appendix Figures 6-15, 6-16**). 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.

2.5 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 *et al.*, 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 (**Appendix Figure 6-17**). *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.

2.6 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 the 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 *et al.*, 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 (**Appendix Figure 6-18**). Interestingly the majority of egg producers were also uncertain about industry efforts (**Appendix Figure 6-19**). 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 (**Appendix Figures 6-20, 6-21**).

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 (**Appendix Figures 6-22, 6-23**). 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.

3 Conclusions and recommendations

In summary:

- 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 (AECL) 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;
- The perceptions of the egg producers and regulators were influenced by the knowledge on the individuals who attended the workshop;
- The continuous dialogue (in the form of workshop or round table discussion) between the industry and regulators is essential. Such dialogue will be helpful in joint discussions and decisions on future research which could address both industry and regulatory issues;
- It is also important to disseminate the findings of research to end users;
- There was a general agreement amongst the producers and regulators that although several on-farm *Salmonella* interventions are available their efficacy is either limited or poorly understood. Future research should be directed at understanding such interventions under controlled or field environment; and
- The epidemiology/ surveillance and virulence of *Salmonella* in supply chain and kitchen environment have been poorly investigated. Future studies should be directed to study the surveillance and the extent of virulence of *Salmonella* spp. along the supply chain.

4 References

AECL (2013) AECL Annual Report. Sydney, Australia: Australian Egg Corporation Limited.

Bachtiar, E. W., Sheng, K. C., Fifis, T., Gamvrellis, A., Plebanski, M., Coloe, P. J., & Smooker, P. M. (2003) Delivery of a heterologous antigen by a registered *Salmonella* vaccine (STM1). *FEMS Microbiol Lett,* 227(2), 211-217.

Chousalkar, K. K., Flynn, P., Sutherland, M., Roberts, J. R., & Cheetham, B. F. (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. doi: 10.1016/j.ijfoodmicro.2010.06.029

Chousalkar, K. K., & Roberts, J. R. (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. doi: 10.3382/ps.2012-02144

Comar, D. (2012) Microbiological safety parameters of raw egg dressings and other raw egg products. (M120017). Melbourne: Silliker Australia Pty Ltd.

Daughtry, B., J., S., G., H., C., T., Grimes, T., R., H., . . . Pointon, A. (2005) National food safety risk profile of eggs and egg products. Sydney, Australia: Australian Egg Corporation Limited.

Davies, R., & Breslin, M. (2003) Environmental contamination and detection of *Salmonella* enterica serovar enteritidis in laying flocks. *Veterinary Record,* 149, 699-704.

Davison, S., Benson, C. E., Henzler, D. J., & Eckroade, R. J. (1999) Field Observations with *Salmonella* enteritidis Bacterins. *Avian Diseases,* 43, 664-669.

De Reu, K., Grijspeerdt, K., Messens, W., Heyndrickx, M., Uyttendaele, M., Debevere, J., & Herman, L. (2006) Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including *Salmonella* enteritidis. *International Journal of Food Microbiology,* 112, 253-260.

Desin, T. S., Koster, W., & Potter, A. A. (2013) *Salmonella* vaccines in poultry: past, present and future. *Expert Rev Vaccines,* 12(1), 87-96. doi: 10.1586/erv.12.138

Douglas, I. (2004). *Salmonella* enteritidis – situation in Queensland. Brisbane.

Fearnley, E., Raupach, J., Lagala, F., & Cameron, S. (2011) *Salmonella* in chicken meat, eggs and humans; Adelaide, South Australia, 2008. *Int J Food Microbiol,* 146(3), 219-227. doi: 10.1016/j.ijfoodmicro.2011.02.004

Galiş, A. M., Marcq, C., Marlier, D., Portetelle, D., Van, I., Beckers, Y., & Théwis, A. (2013) Control of *Salmonella* Contamination of Shell Eggs—Preharvest and Postharvest Methods: A Review. *Comprehensive Reviews in Food Science and Food Safety, 12,* 155-182.

Gast, R. K. (2007). Serotype-specific and serotype-independent strategies for preharvest control of food-borne *Salmonella* in poultry. *Avian Dis,* 51(4), 817-828.

Gast, R. K., Guraya, R., Guard-Bouldin, J., & Holt, P. S. (2007). In vitro penetration of egg yolks by *Salmonella* Enteritidis and *Salmonella* Heidelberg strains during thirty-six-hour ambient temperature storage. *Poultry Science,* 86, 1431-1435.

Gast, R. K., & Holt, P. S. (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.

Gast, R. K., Holt, P. S., & Guraya, R. (2006) Effect of refrigeration on in vitro penetration of *Salmonella* Enteritidis through the egg yolk membrane. *Journal of Food Protection*, 69, 1426-1429.

Gole, V. C., Chousalkar, K. K., Roberts, J. R., Sexton, M., May, D., Tan, J., & Kiermeier, A. (2014) Effect of egg washing and correlation between eggshell characteristics and egg penetration by various *Salmonella* Typhimurium strains. PLoS One, 9(3), e90987. doi: 10.1371/journal.pone.0090987

Gole, V. C., Roberts, J. R., Sexton, M., May, D., Kiermeier, A., & Chousalkar, K. K. (2014). Effect of egg washing and correlation between cuticle and egg penetration by various *Salmonella* strains. *Int J Food Microbiol,* 182-183, 18-25. doi: 10.1016/j.ijfoodmicro.2014.04.030

Gole, V. C., Torok, V., Sexton, M., Caraguel, C. G., & Chousalkar, K. K. (2014). Association between indoor environmental contamination by *Salmonella* enterica and contamination of eggs on layer farms*. J Clin Microbiol,* 52(9), 3250-3258. doi: 10.1128/JCM.00816-14

Groves, P. J., Sharpe, S. M., & Cox, J. M. (2015) Response of layer and broiler strain chickens to parenteral administration of a live *Salmonella* Typhimurium vaccine. *Poult Sci,* 94(7), 1512-1520. doi: 10.3382/ps/pev127

Hutchison, M. L., Gittins, J., Sparks, A. W., Humphrey, T. J., Burton, C., & Moore, A. (2004) An assessment of the microbiological risks involved with egg washing under commercial conditions. *J Food Prot,* 67(1), 4-11.

Jones, D. R., Lawrence, K. C., Yoon, S. C., & Heitschmidt, G. W. (2011) *Salmonella* contamination in shell eggs exposed to modified-pressure imaging for microcrack detection. *Poult Sci,* 90(7), 1616-1619. doi: 10.3382/ps.2010-01155

Kinde, H., Castellan, D. M., Kerr, D., Campbell, J., Breitmeyer, R., & Ardans, A. (2005) Longitudinal Monitoring of Two Commercial Layer Flocks and Their Environments for *Salmonella* enterica Serovar Enteritidis and Other Salmonellae. *Avian Diseases,* 49, 1189-1194.

Luber, P. (2009) Cross-contamination versus undercooking of poultry meat or eggs - which risks need to be managed first? *Int J Food Microbiol,* 134(1-2), 21-28. doi: 10.1016/j.ijfoodmicro.2009.02.012

Martelli, F., & Davies, R. H. (2012) *Salmonella* serovars isolated from table eggs: An overview. *Food Research International,* 45, 745-754.

McWhorter, A. R., & Chousalkar, K. K. (2015) Comparative phenotypic and genotypic virulence of *Salmonella* strains isolated from Australian layer farms. *Front Microbiol,* 6, 12. doi: 10.3389/fmicb.2015.00012

McWhorter, A. R., Davos, D., & Chousalkar, K. K. (2015) Pathogenicity of *Salmonella* strains isolated from egg shells and the layer farm environment in Australia. *Appl Environ Microbiol,* 81(1), 405-414. doi: 10.1128/AEM.02931-14

Miyamoto, T., Horie, T., Baba, E., Sasai, K., Fukata, T., & Arakawa, A. (1998) *Salmonella* penetration through eggshell associated with freshness of laid eggs and refrigeration. *J Food Prot,* 61(3), 350-353.

Octavia, S., Wang, Q., Tanaka, M. M., Kaur, S., Sintchenko, V., & Lan, R. (2015) Delineating community outbreaks of *Salmonella* enterica serovar Typhimurium by use of whole-genome sequencing: insights into genomic variability within an outbreak. *J Clin Microbiol,* 53(4), 1063-1071. doi: 10.1128/JCM.03235-14

OzFoonet (2010) Monitoring the incidence and causes of diseases potentially transmitted by food in Australia. Canberra, ACT.

OzFoonet (2012) Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: Annual report of the Ozfoodnet network, 2010. Canberra, ACT.

Patil, S. R., Cates, S., & Morales, R. (2005) Consumer food safety knowledge, practices, and demographic differences: findings from a meta-analysis. *J Food Prot,* 68(9), 1884-1894.

Patterson, S. K., Borewicz, K., Johnson, T., Xu, W., & Isaacson, R. E. (2012) Characterization and differential gene expression between two phenotypic phase variants in Salmonella enterica serovar Typhimurium. *PLoS One,* 7(8), e43592. doi: 10.1371/journal.pone.0043592

Sauter, E. A., Petersen, C. F., Parkinson, J. F., & Steele, E. E. (1979) Effect of pH on eggshell penetration by *Salmonellae*. *Poult Sci,* 58(1), 135-138.

Stephens, N., Coleman, D., & Shaw, K. (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.

Stephens, N., Sault, C., Firestone, S. M., Lightfoot, D., & Bell, C. (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.

Van Hoorebeke, S., Van Immerseel, F., Haesebrouck, F., Ducatelle, R., & Dewulf, J. (2011) The influence of the housing system on Salmonella infections in laying hens: a review. *Zoonoses Public Health,* 58(5), 304-311. doi: 10.1111/j.1863-2378.2010.01372.x

Van Immerseel F, Russell JB, Flythe MD, Gantois I, Timbermont L, Pasmans F, R., D. (2006) The use of organic acids to combat Salmonella in poultry: a mechanistic explanation of the efficacy. avian Pathology, 35, 182-188.

Waimaleongora-Ek, P., Garcia, K. M., No, H. K., Prinyawiwatkul, W., & Ingram, D. R. (2009) Selected quality and shelf life of eggs coated with mineral oil with different viscosities*. J Food Sci,* 74(9), S423-429. doi: 10.1111/j.1750-3841.2009.01341.x

Wales, A. D., Berslin, M., Carter, B., Sayers, R., & Davis, R. (2007). A longitudinal study of environmental *Salmonella* contamination in caged and free range layer flocks. *Avian Pathology*, 36, 187-197.

5 Plain English Summary

Project Title:	A workshop for development of Salmonella control strategies in egg industry
AECL Project No	1UA141
Researchers Involved	K. Chousalkar, M. Sexton and A. McWhorter
Organisations Involved	The School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy, 5317, SA, Australia
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Objectives	1 To initiate discussion between egg industry and regulatory authorities, discuss on-farm risk factors and intervention strategies on and off the egg farm. 2 To obtain information from participants about their understanding of issues to help the egg industry funding body to focus on priority R&D areas. 3 To provide evidence based information to regulators and egg producers, review current practises on farm and also in supply chain. 4 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.
Background	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 FSANZ. 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.
Research	In this report the current evidence-based information and workshop discussions are presented. The survey results collected from the workshop participants are also presented. 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.

Project Title:	A workshop for development of Salmonella control strategies in egg industry
Outcomes	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.
Implications	• The continuous dialogue (in form of workshop or round table discussion) between the industry and regulators is essential. Such dialogue will be helpful in joint discussions and decisions on future research which could address both industry and regulatory issues. • Future work is required to understand the *Salmonella* interventions in field and controlled experimental conditions. There is also a need to study the epidemiology and virulence of *Salmonella* in supply chain.
Key Words	Salmonella, workshop
Publications	This report has been published in Critical Reviews in Food Science and Nutrition

6 Appendix

The following figures present the workshop survey results.

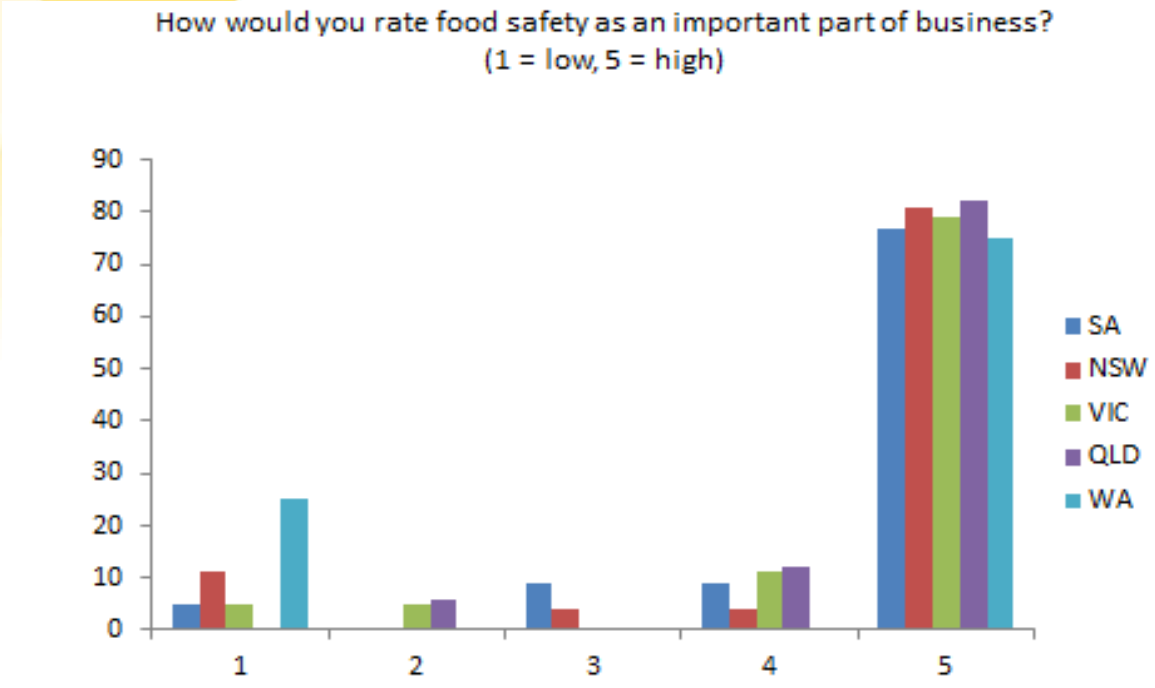

Figure 6-1: Workshop survey results for Question 1a - How would you rate food safety as an important part of business?

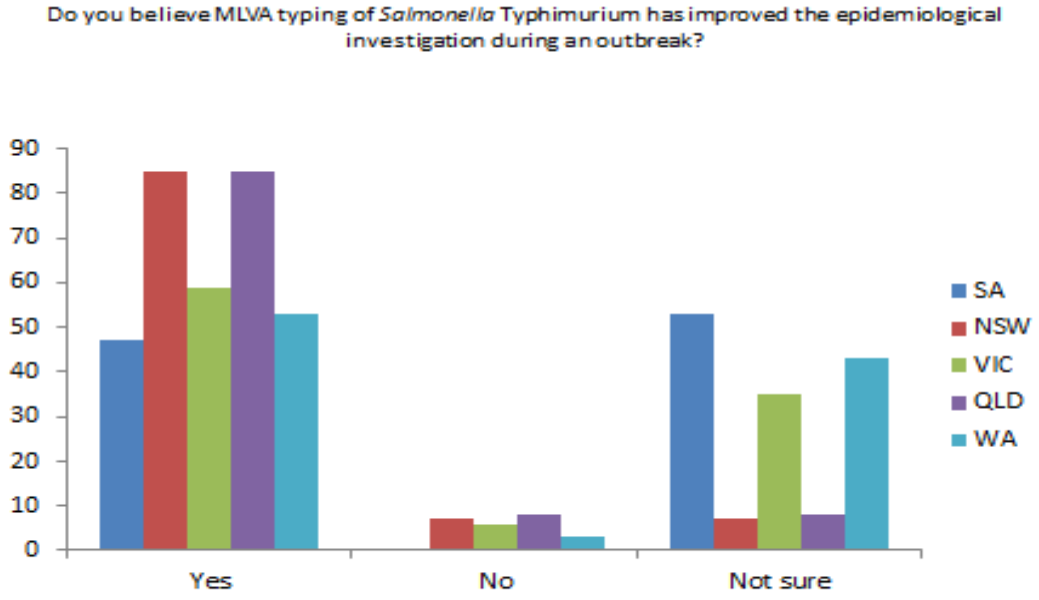

Figure 6-2: Workshop survey results for Question 1b - Do you believe MLVA typing of *Salmonella* Typhimurium has improved the epidemiological investigation during an outbreak?

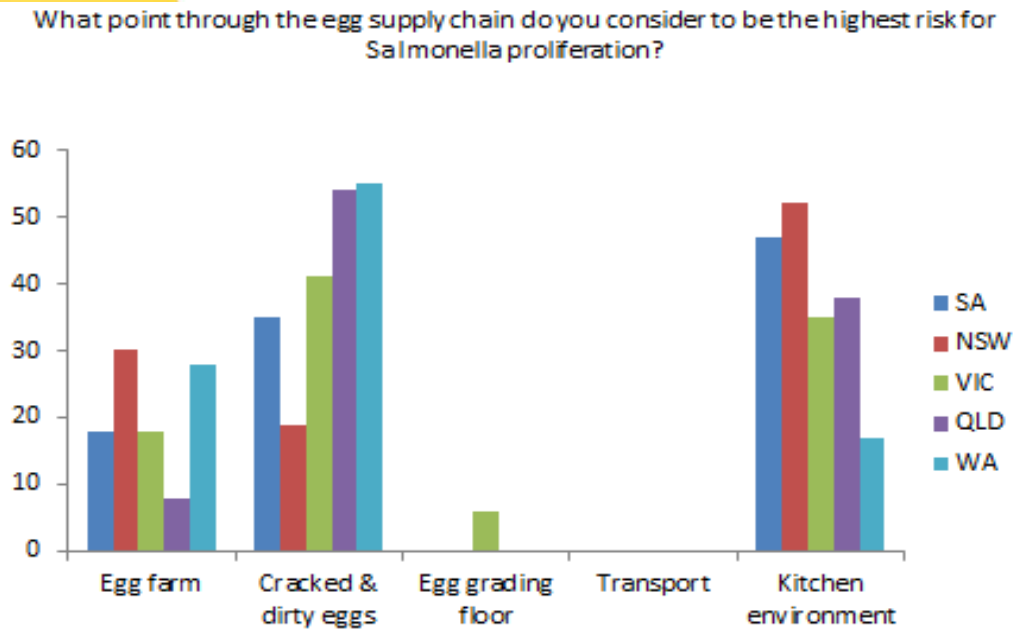

Figure 6-3: Workshop survey results for Question 1c – What point through the egg supply chain do you consider to be the highest risk for *Salmonella* proliferation?

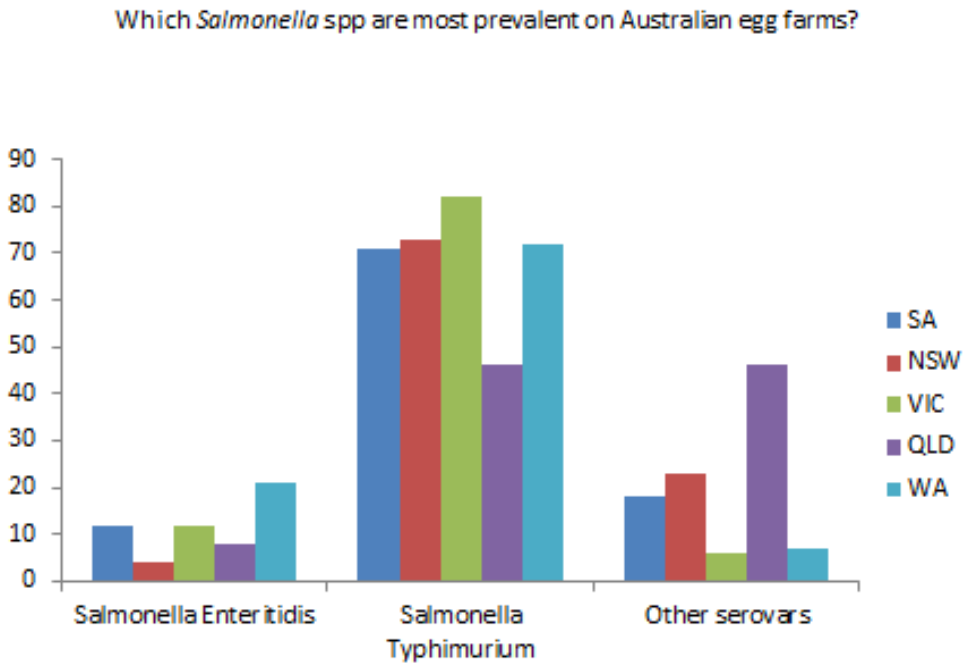

Figure 6-4: Workshop survey results for Question 1d – Which *Salmonella* spp. are most prevalent on Australian egg farms?

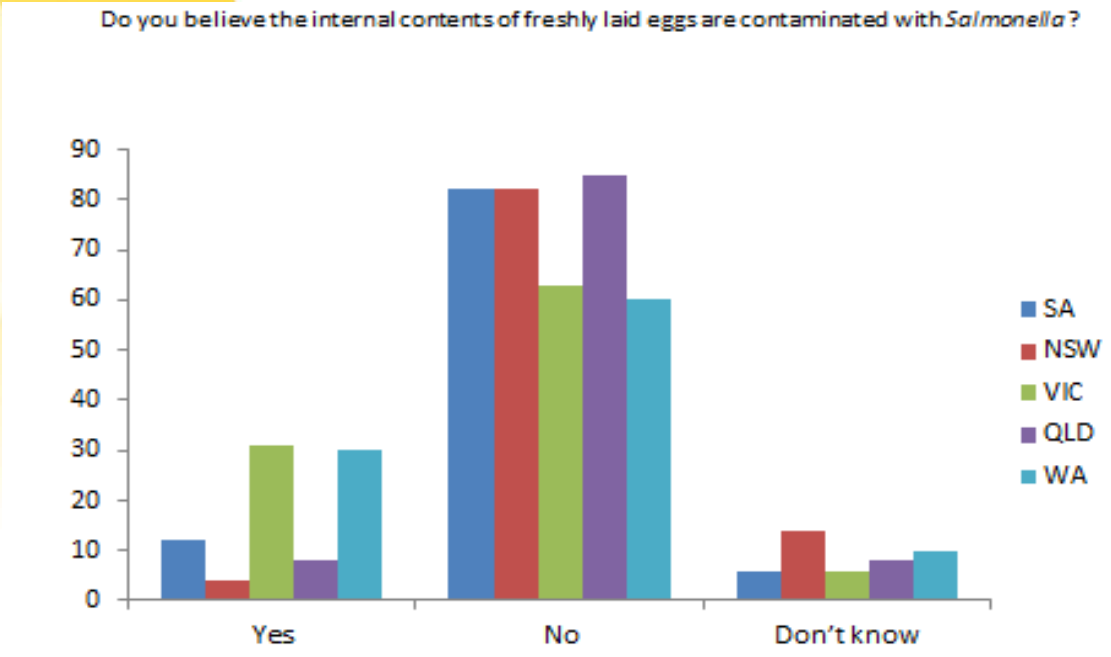

Figure 6-5: Workshop survey results for Question 1e – Do you believe the internal contents of freshly laid eggs are contaminated with *Salmonella?*

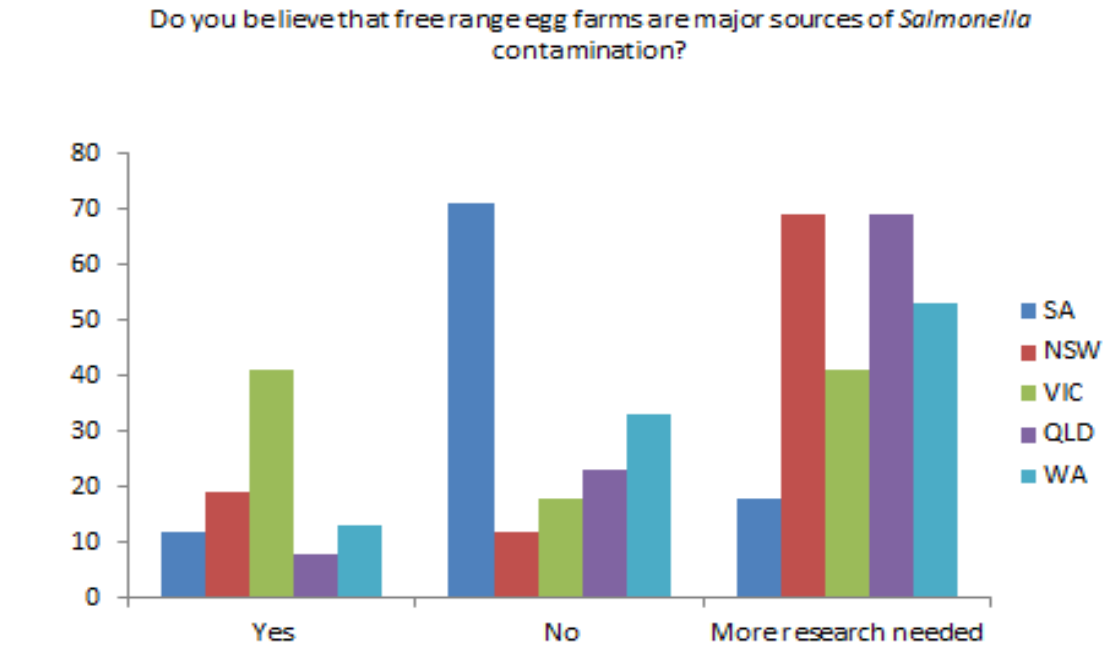

Figure 6-6: Workshop survey results for Question 1f – Do you believe that free range egg farms are major sources of *Salmonella* contamination?

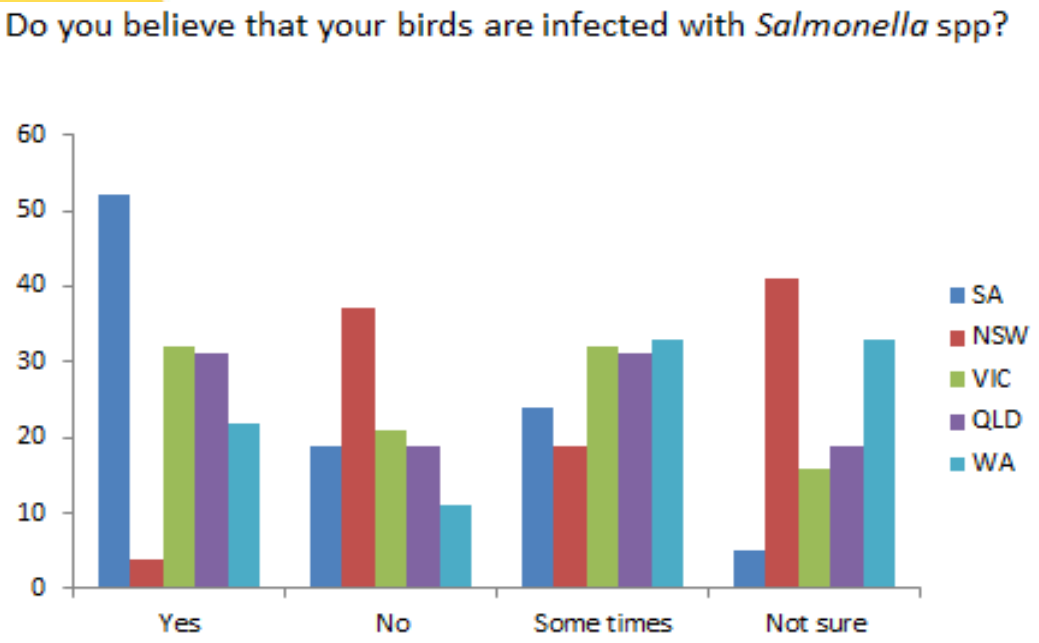

Figure 6-7: Workshop survey results for Question 1g – Do you believe that your birds are infected with *Salmonella* spp.?

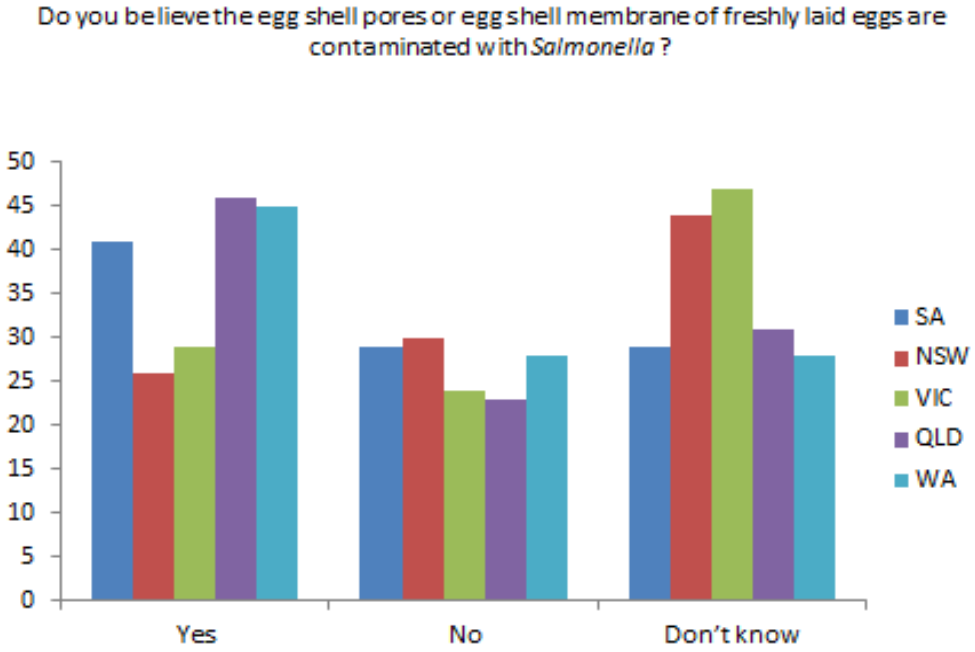

Figure 6-8: Workshop survey results for Question 1h – Do you believe the egg shell pores or egg shell membrane of freshly laid eggs are contaminated with *Salmonella?*

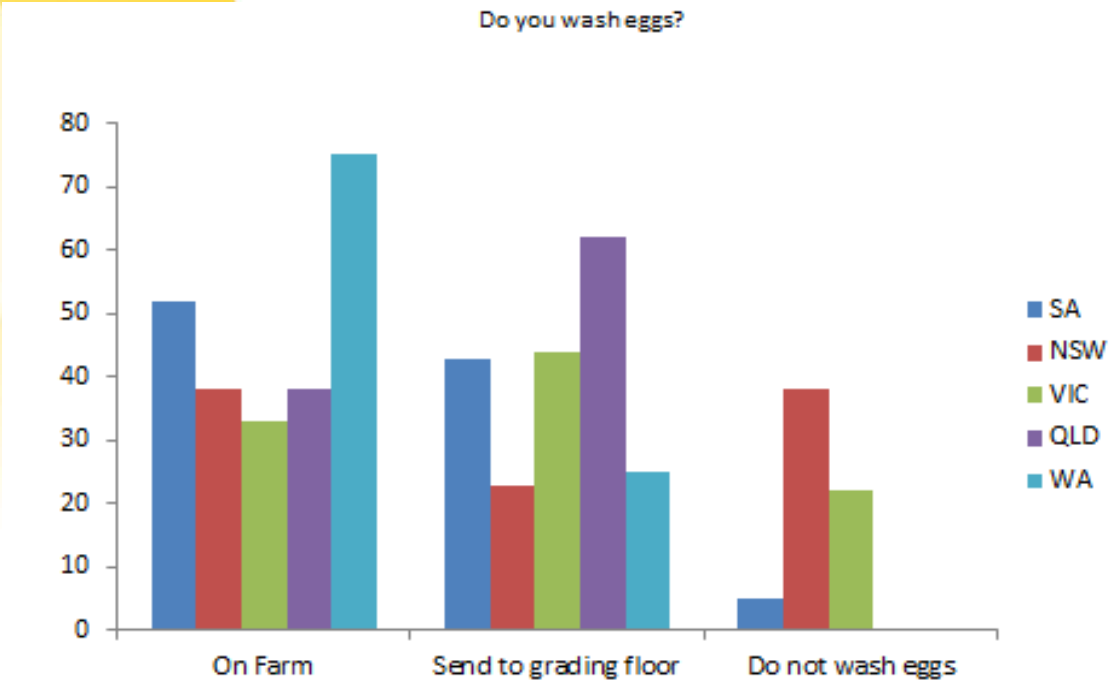

Figure 6-9: Workshop survey results for Question 1i – Do you wash eggs?

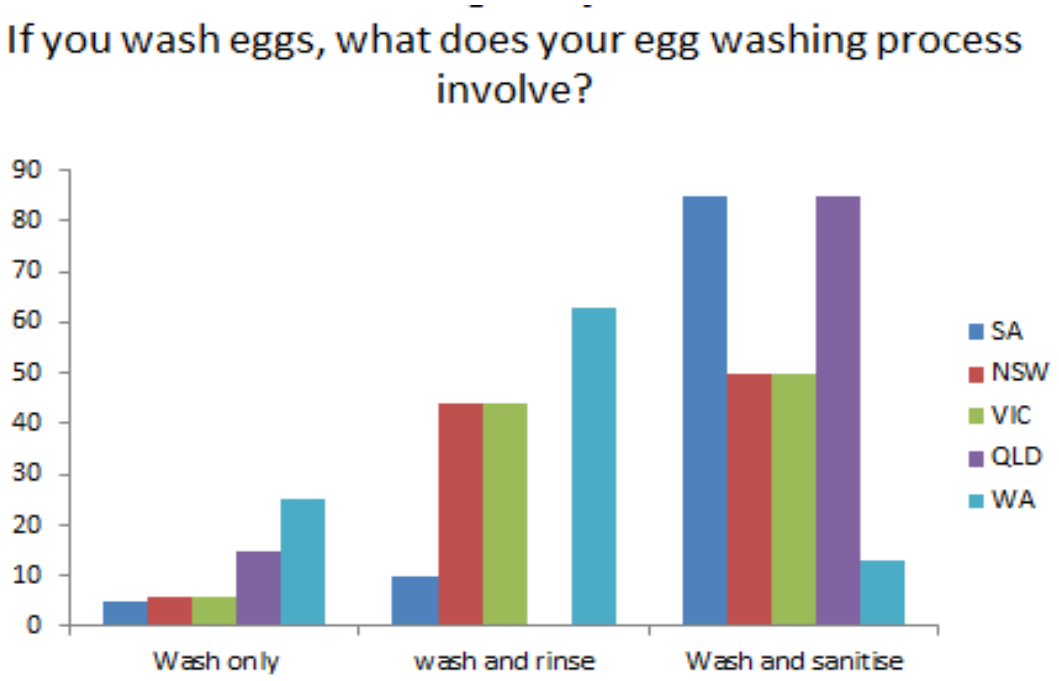

Figure 6-10: Workshop survey results for Question 1j – If you wash eggs, what does your egg washing process involve?

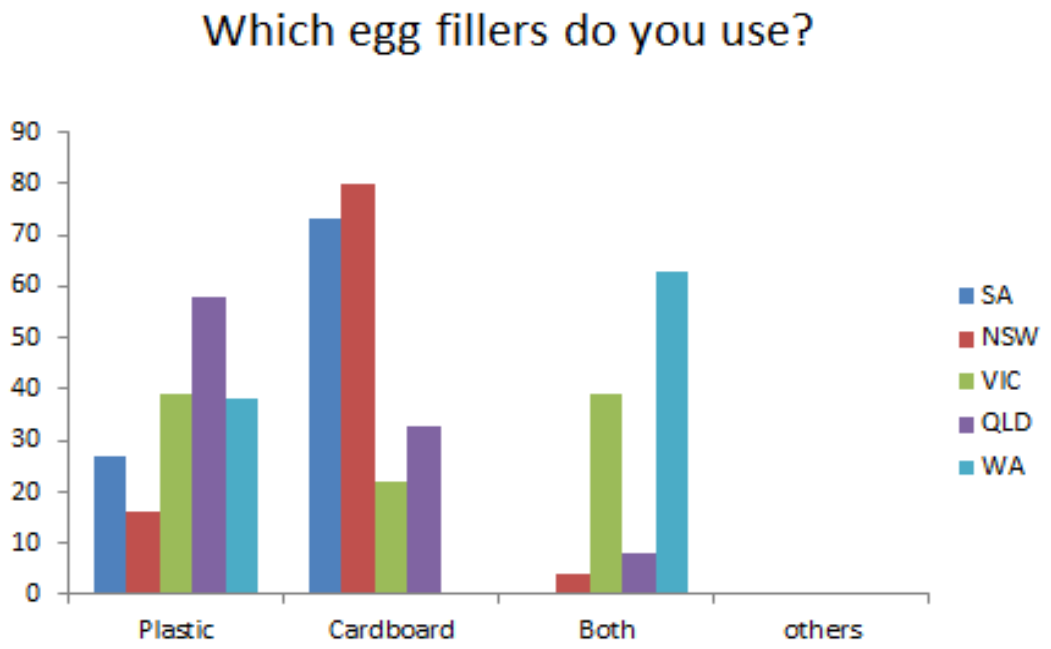

Figure 6-11: Workshop survey results for Question 1k – Which egg fillers do you use?

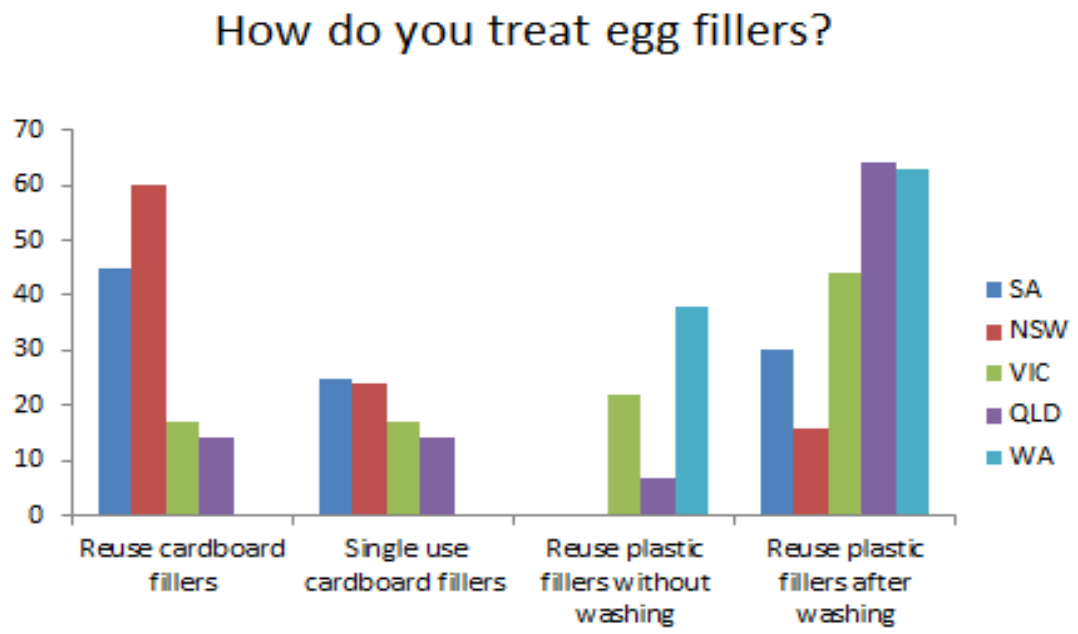

Figure 6-12: Workshop survey results for Question 1i – How do you treat egg fillers?

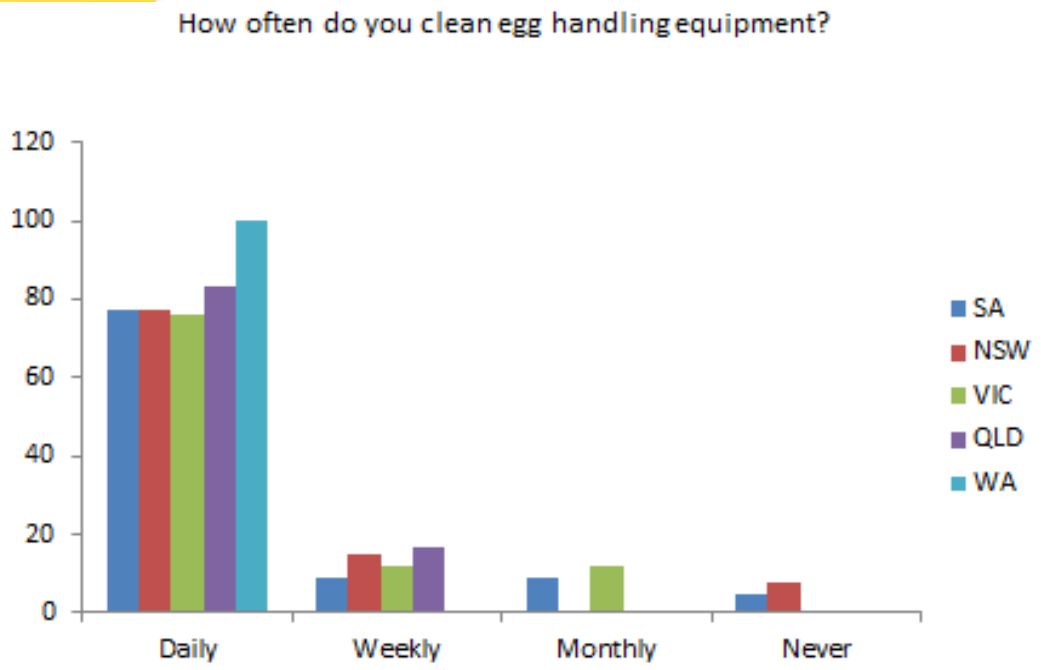

Figure 6-13: Workshop survey results for Question 1m – How often do you clean egg handling equipment?

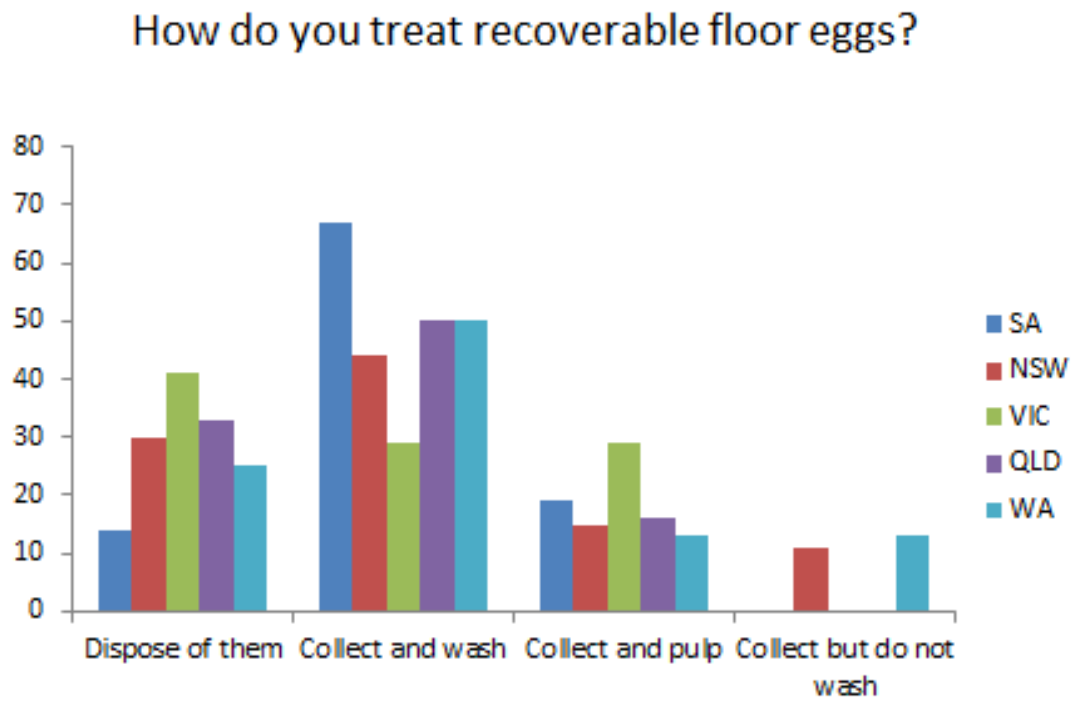

Figure 6-14: Workshop survey results for Question 1n – How do you treat recoverable floor eggs?

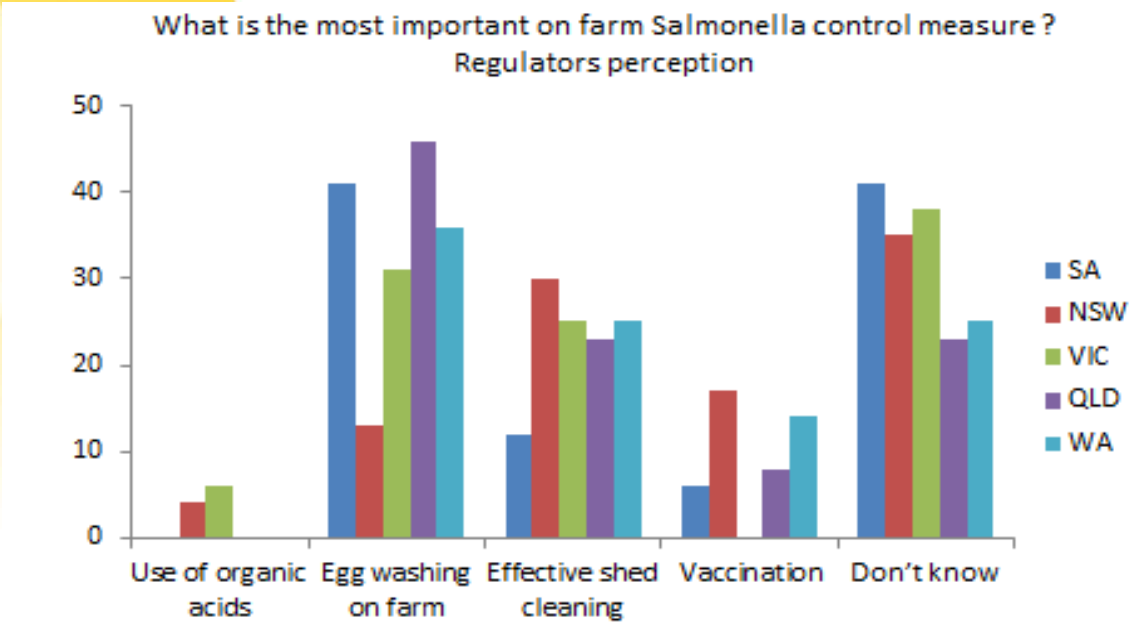

Figure 6-15: Workshop survey results for Question 1o – What is the most important on-farm *Salmonella* control measure? Regulators perception.

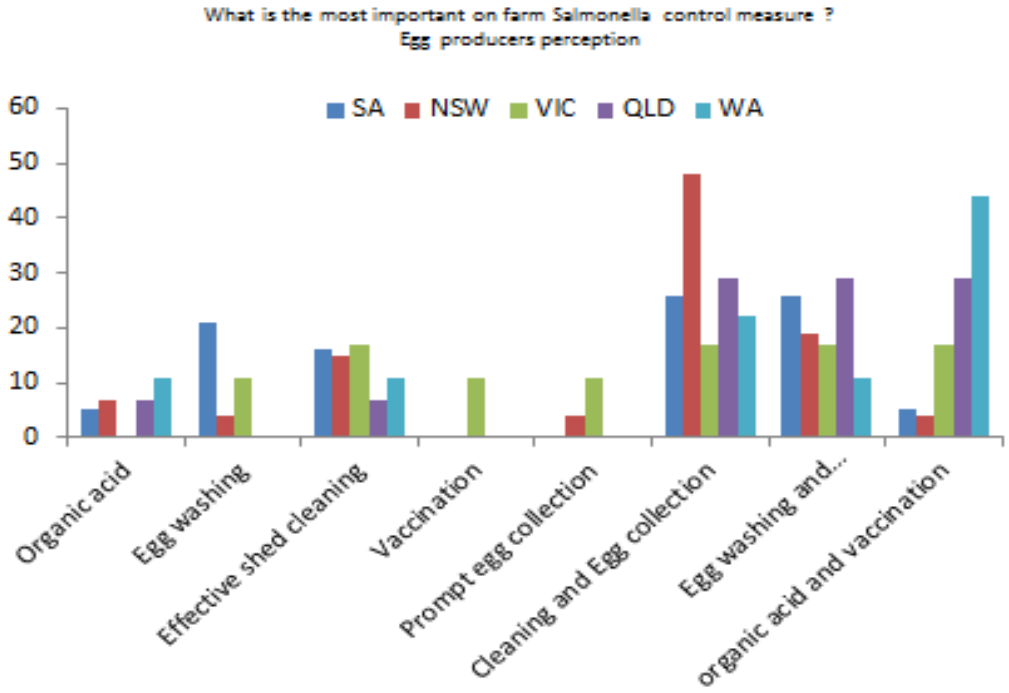

Figure 6-16: Workshop survey results for Question 1p – What is the most important on-farm *Salmonella* control measure? Egg producers' perception.

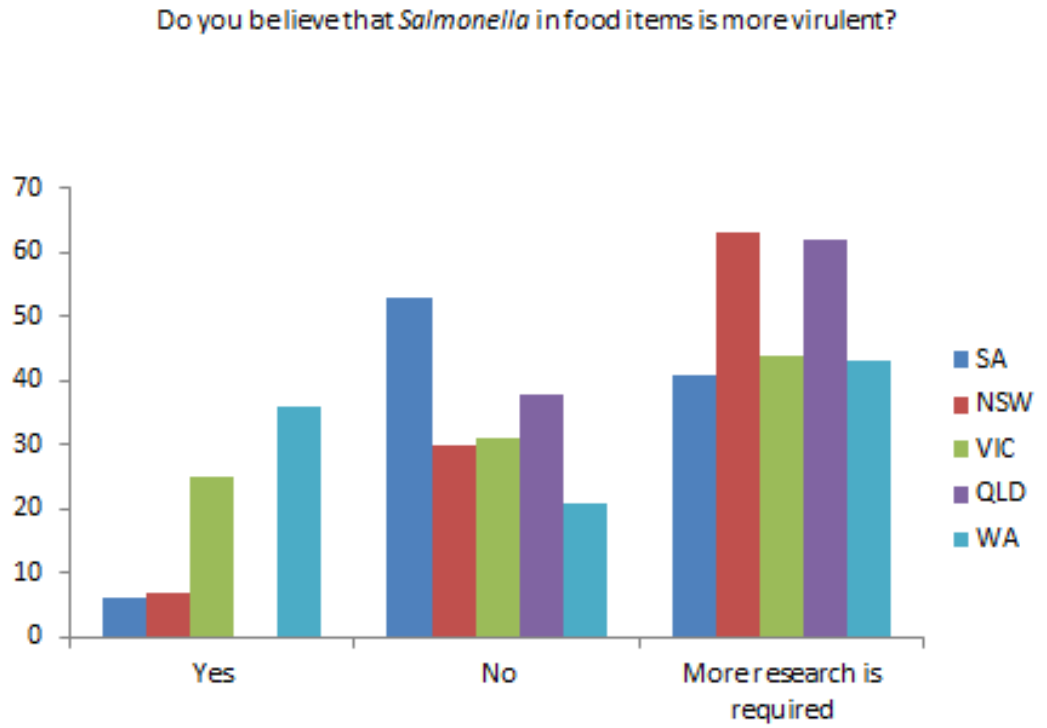

Figure 6-17: Workshop survey results for Question 1q – Do you believe that *Salmonella* in food items is more virulent?

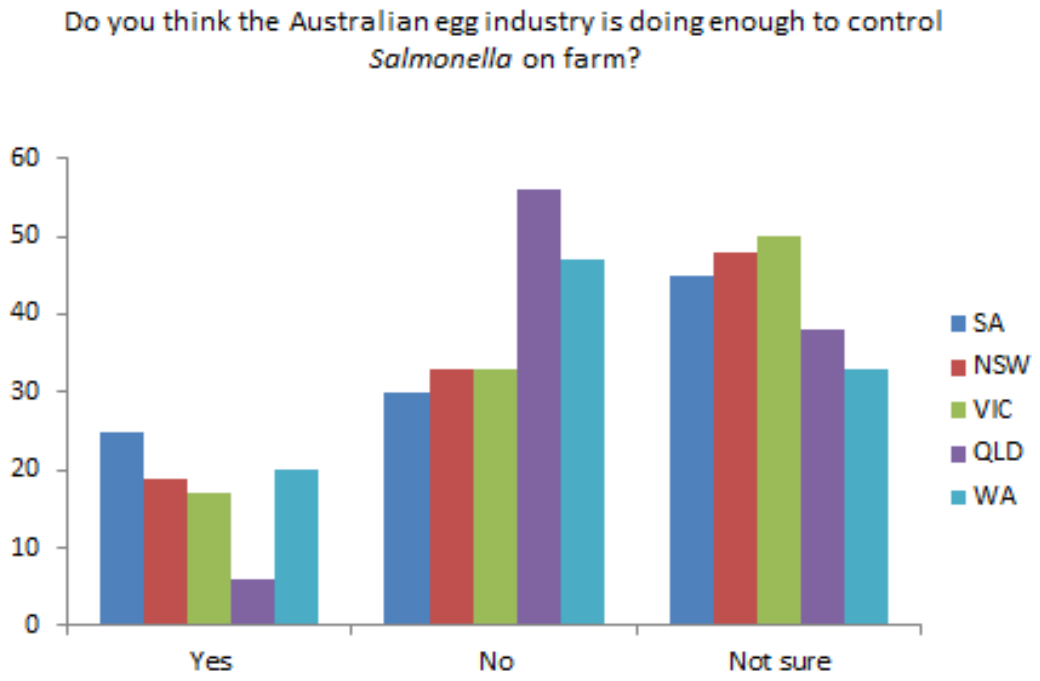

Figure 6-18: Workshop survey results for Question 1r – Do you think the Australian egg industry is doing enough to control *Salmonella* on-farm?

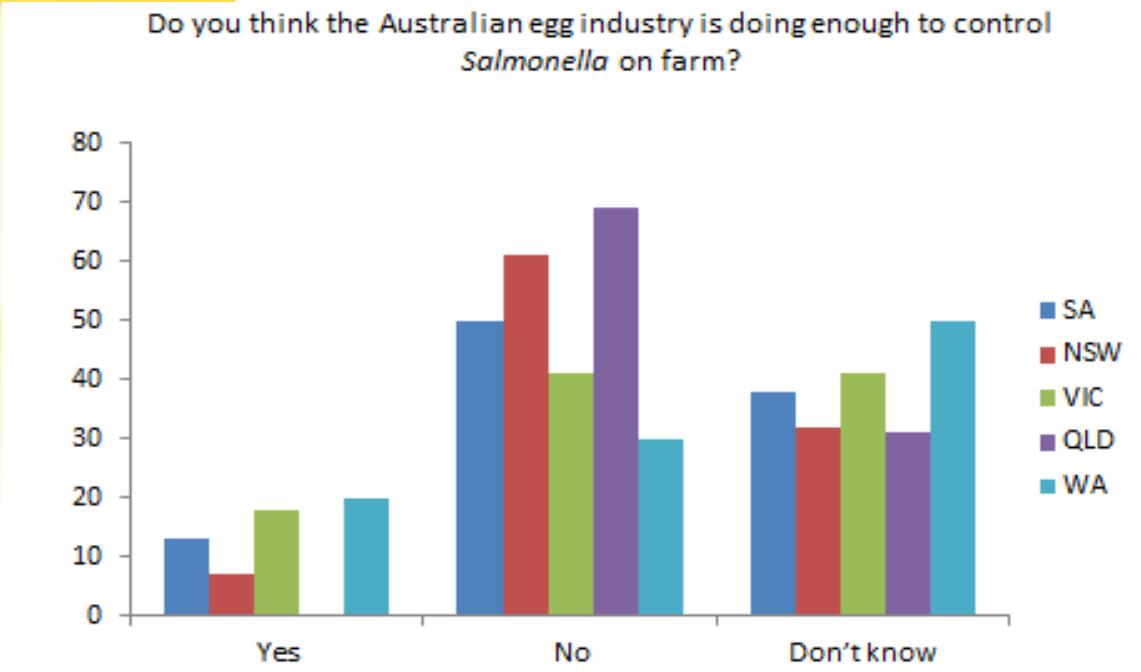

Figure 6-19: Workshop survey results for Question 1s – Do you think the Australian egg industry is doing enough to control *Salmonella* on-farm?

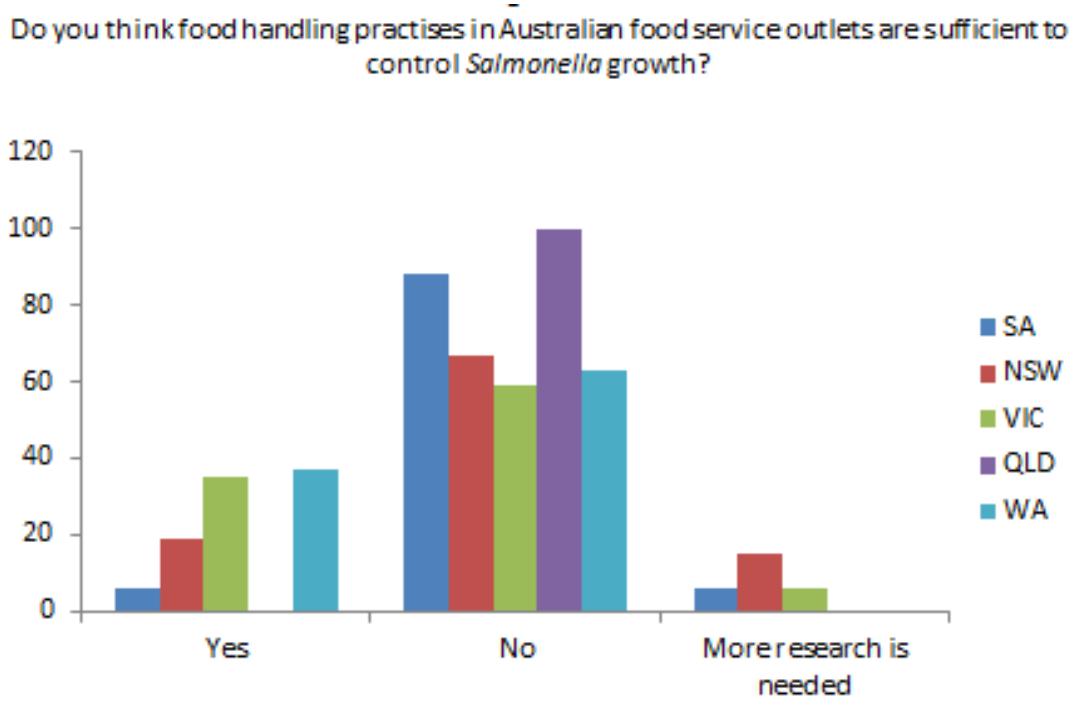

Figure 6-20: Workshop survey results for Question 1t – Do you think food handling practices in Australian food service outlets are sufficient to control *Salmonella* growth?

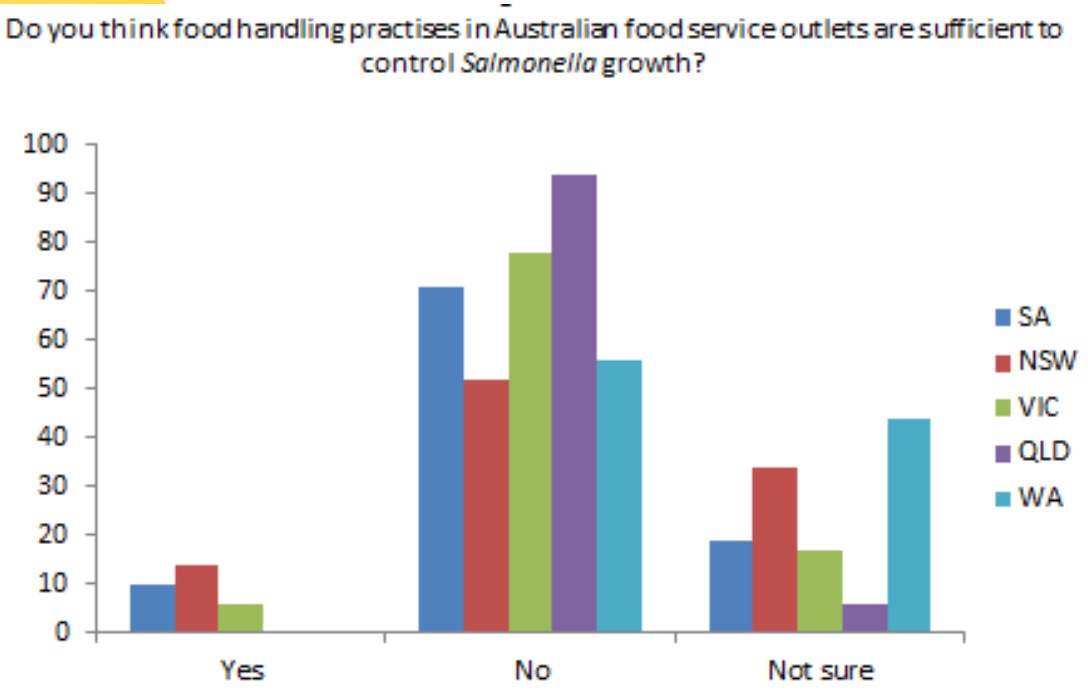

Figure 6-21: Workshop survey results for Question 1u – Do you think food handling practices in Australian food service outlets are sufficient to control *Salmonella* growth?

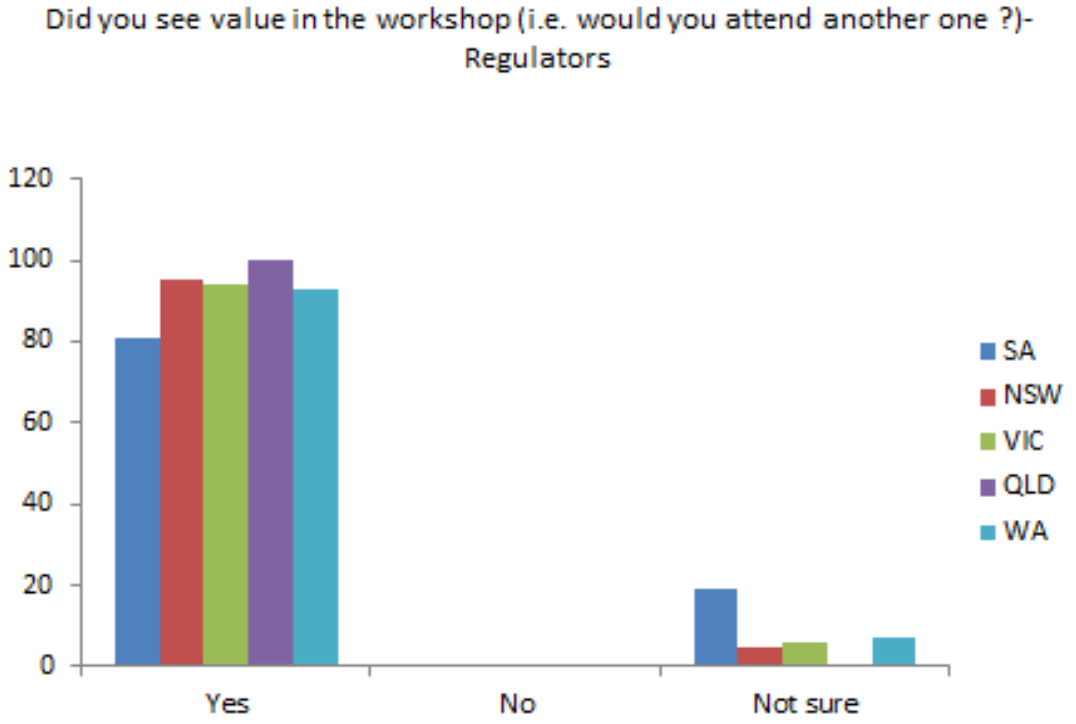

Figure 6-22: Workshop survey results for Question 1v – Did you see value in the workshop (i.e. would you attend another one?)? Regulators.

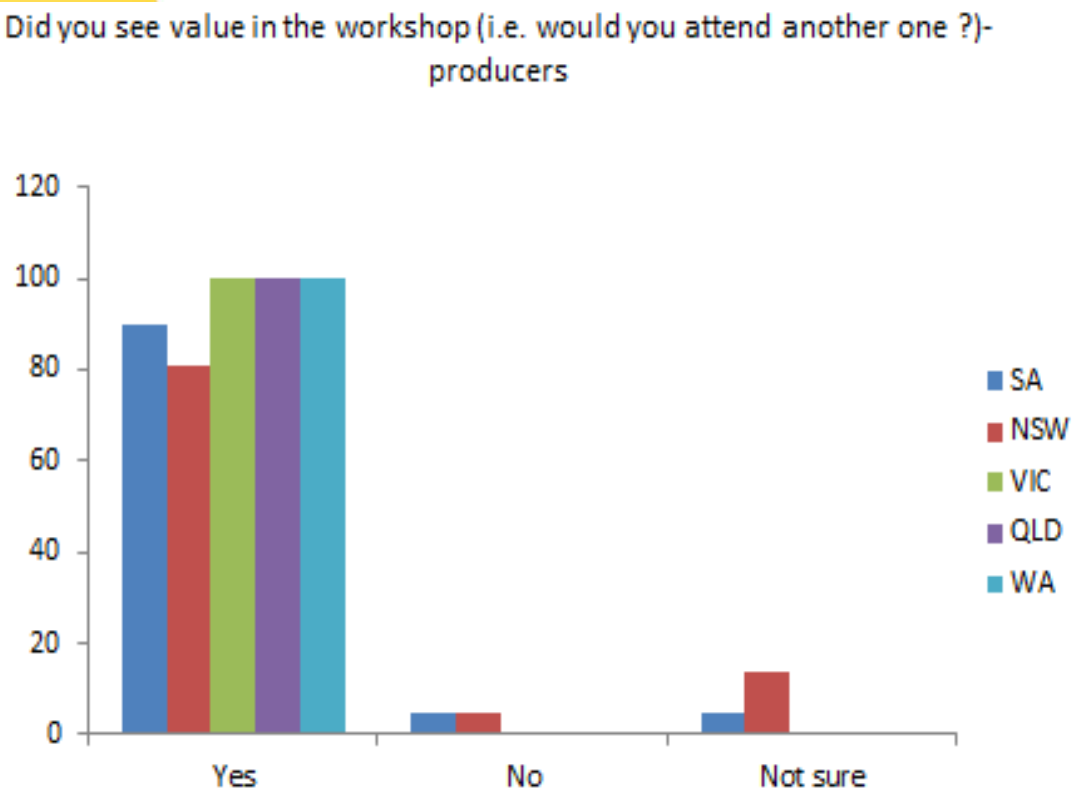

Figure 6-23: Workshop survey results for Question 1w – Did you see value in the workshop (i.e. would you attend another one)? Producers.