NSW GOVERNMENT Food Authority

Baseline evaluation of the NSW Egg Food Safety Scheme

Microbiological survey of egg farms in NSW

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About this document

This document reports the microbiological baseline survey results of egg businesses in NSW.

It is one of two reports that have been prepared as part of the baseline evaluation study of the NSW Egg Food Safety Scheme undertaken by the NSW Food Authority in 2010–11.

Survey results of industry profile and observed practices are reported in the *Baseline evaluation of the NSW Egg Food Safety Scheme*: *Survey of NSW egg businesses – industry profile and observed practices (2012).*

If you have any questions about this document, please contact the NSW Food Authority helpline on 1300 552 406 or contact@foodauthority.nsw.gov.au.

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Laboratory analysis was conducted by NSW Forensic and Analytical Science Services, Lidcombe, Institute for Clinical Pathology and Medical Research, Westmead, and IMVS Pathology, Adelaide.

Contents

Executive summary

Overview

In June 2010, the Egg Food Safety Scheme (Egg Regulation[1]) was introduced in NSW. Initial regulatory visits of egg businesses commenced four months later in October 2010.

The Egg Regulation covers businesses producing, grading or processing eggs and egg products for sale. Under the Regulation, egg businesses are required to be licensed with the NSW Food Authority (the Authority) if they produce or grade more than twenty dozen eggs for sale in any week.

By 30 November 2011, there were 199 licensed egg businesses comprising:

- 74 licensed egg primary production businesses (egg producers), and
- 125 licensed egg primary production businesses with additional activities such as grading and washing (egg producers/graders).

The Authority undertook a number of activities supporting the implementation of the Egg Regulation. This included preparing industry-specific assistance materials and conducting specialist training for Authority officers engaged in the audit and inspection program. At the first regulatory visit, all licensed egg businesses received advice from Authority officers regarding the new requirements.

In addition to gathering industry profile (eg production system, flock size, egg volume) and food safety information at the initial regulatory visit[2], Authority officers also collected samples for microbiological testing from approximately 30% (49/165) of egg businesses inspected or audited between 1 December 2010 and 30 November 2011. Overall, this represented one-quarter of all licensed egg businesses at the time[3].

The microbiological survey of egg producers (egg farms) in NSW intended to:

1. provide the Authority with an up-to-date summary of egg industry risks by estimating prevalence of Salmonella on egg farms in NSW including the establishment of a microbiological profile of *Salmonella* serovars for comparison with notified cases of human salmonellosis in NSW,
2. establish useful microbiological benchmarks for *Salmonella* in the egg laying environment and farm/shed inputs (eg stock feed and drinking water) and *E. coli* (water only) which can serve as a point of reference for assessing the impact of the Egg Regulation in the future, and
3. collect data which provides insight into the appropriateness of the current Egg Regulation requirements by identifying areas of potential concern at the initial regulatory visit. This can inform development of industry assistance and regulatory (audit/inspection) assistance that are most useful for businesses.

Methods

More than 380 environmental (boot/cage swabs and faecal material) and farm input (stock feed and drinking water) samples were collected from 49 farms. For each farm, samples were collected from a maximum of four sheds. From each shed, a set of four samples was collected comprising boot/cage swab, faecal material, feed at point of consumption and hen drinking water. For farms with less than four sheds, each shed was sampled as described. If available, samples of bulk stored feed and drinking water source samples were also taken from each farm. Participation in the survey was voluntary. To ensure state-wide coverage, proportionate numbers of farms were randomly selected from twelve regional areas in NSW.

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[1] A Food Safety Scheme under the NSW Food Regulation 2010

[2] NSW Food Authority (2012), *Baseline evaluation of the NSW Egg Food Safety Scheme: Survey of NSW egg businesses – industry profile and observed practices (2012).* Available at: www.foodauthority.nsw.gov.au

[3] Businesses licensed by 30 November 2011 (n=199)

All samples were analysed for serovars of *Salmonella.* Some water samples were also tested for *E. coli*. Overall *Salmonella* prevalence was calculated for farm/shed inputs (stock feed and water) and egg laying environment.

Further data analysis considered *Salmonella* prevalence by production system, egg production volume, flock age, season and location. However, it is worth noting that based on the level of sampling undertaken for this survey, statistically valid conclusions cannot be made when assigning cause to any observed differences noted for these categories.

A farm, shed or flock was categorised as 'positive' if at least one sample was positive for *Salmonella* and negative if all samples for the farm, shed or flock were negative. The results distinguish between *Salmonella* serovar and *S.* Typhimurium where relevant. *S.* Typhimurium isolates were analysed further via phage and MLVA typing.

General findings

Survey findings are summarised as follows:

- Of the 49 egg farms in the survey, just under half (22/49) were positive for *Salmonella*. Specifically, 20% (10/49) of farms were positive for *S.* Typhimurium. No farm in the NSW survey was positive for *S.* Enteritidis.
- Overall, *Salmonella* prevalence was higher for egg laying environment samples (boot/cage swabs and faecal material) than samples of farm/shed inputs (stock feed and drinking water).
- *Farm level inputs:*
    - For bulk stored feed, 11% (3/27) samples were positive for *Salmonella. S.* Typhimurium was not detected in any bulk stored feed sample.
    - For bulk stored feed, *Salmonella* prevalence of self-produced feed was similar (14%, 1/7) to purchased feed (10%, 2/18).
    - None (0/20) of the drinking water source samples (reticulated and non-reticulated) were positive for *Salmonella,* but half (5/10) of the samples (all non-reticulated water) that underwent additional analysis contained detectable levels of *E. coli* indicating faecal contamination.
- *Shed level inputs:*
    - Due to increased risk of cross-contamination from the shed environment, higher *Salmonella* prevalence was found in feed at point of consumption and hen drinking water than for bulk stored feed and water source samples.
    - In total, 17% (17/101) of feed at point of consumption and 6% (3/46) of hen drinking water samples tested positive for *Salmonella*.
- *Egg laying environment:*
    - In the egg laying environment, sample analysis found that just over one quarter (26/99) of boot/cage swabs were positive for *Salmonella* and prevalence of *S*. Typhimurium was 10% (9/99).
    - *Salmonella* prevalence for faecal material was lower (17%, 15/90) compared with boot/cage swabs (above). In total, 9% (8/90) of all faecal material samples were positive for *S.* Typhimurium.
- The number of farms with positive *Salmonella* samples ('positive farms'), and the proportion of positive samples on those farms, provides overall benchmarks for monitoring the impact of the Regulation in the future. The proportion of positive samples provides a general indication of the overall effectiveness of food safety management practices. Overall, for 27 farms, *Salmonella* was not isolated from any sample. For 'positive farms' selected percentile rankings were calculated as follows:

- For half of the *Salmonella* positive farms (at the 50th percentile), at most, the proportion of positive *Salmonella* samples was 27%.
- For 95% of the *Salmonella* positive farms (at the 95th percentile), at most, two-thirds of samples were positive.
- For 99% of positive farms, at most (at the 99th percentile), 93% of samples were positive.
- Over time, as egg farms progress with their implementation of the Egg Regulation requirements, the Authority expects to see fewer positive sample sites on farms and an increased proportion of farms with all negative samples.

- In total, seventeen serovars were isolated across the *Salmonella* positive egg farms in the survey. *S.* Typhimurium was the predominant serovar accounting for 30% (39/130) of all the *Salmonella* positive samples, followed by *S.* Infantis (19%, 25/130).
- In 2011, there were two serovars that were common to both farms and notified human salmonellosis cases: *S.* Typhimurium and *S.* Infantis.
- In total, six *S.* Typhimurium phage types were identified from the surveyed egg farms in NSW. Four out of the five most frequently isolated phage types in notified human cases in 2011 were also identified on the egg farms in this survey.
- *S.* Typhimurium MLVA analysis of egg farm samples identified seven MLVA types. Two types were common to notified human cases. *S.* Typhimurium MLVA 3-9-7-13-523 was the most frequently detected MLVA type, both in this study and isolated in humans, in NSW in 2011. *S.* Typhimurium MLVA 3-9-7-15-523 was the only other MLVA type in common.

Conclusion

The ultimate aim of the Egg Regulation is to reduce the incidence and potential for foodborne illness from eggs and egg products. This survey provides clear justification for the introduction of regulatory food safety measures for the egg industry in NSW since *Salmonella* was detected on close to half of the farms surveyed. Many of the *Salmonella* types that predominated on farms also predominate amongst isolates from humans. While there is sufficient evidence in the scientific literatures to show that the presence of *Salmonella* in the egg laying environment does not automatically infer a high prevalence on whole eggs offered for sale, it does highlight increased risk associated with cross contamination of *Salmonella* from the environment to whole eggs.

As best practice implementation, survey data was used to benchmark *Salmonella* prevalence in egg laying environments and farm inputs and *E. coli* (water only) for farms in NSW. Data provides a point of comparison for assessing future impacts of the Egg Regulation and for monitoring any changes to composition and activities of the NSW egg industry.

The survey findings have also provided insight into the appropriateness of Egg Regulation requirements by highlighting areas of potential concern at the initial regulatory visit. In future, the Authority plans to work with industry to ensure that risk management approaches remain targeted and effective, especially in relation to reducing:

- flock-to-flock transmission of *Salmonella* ,
- contamination risks for feed and feed ingredients, and
- potential for contamination of drinking water (water source and hen drinking water).

Due to the lack of published scientific literature for Australian egg farms, the survey data also provides a solid evidence base upon which to build future findings. However, when evaluating the impact of the Egg Regulation in the future, as a matter of practice, the Authority will consider other measures in addition to the apparent prevalence of *Salmonella* on farms. Other strategies for examining the causal impacts of the Regulation are to be included as it is not unusual for microbiological surveys of this kind to be subjected to a number of confounding factors including the effect of time, seasonality and flock age.

1. Establishing a microbiological profile of egg farms in NSW is a good starting point for effective regulation

The NSW Egg Food Safety Scheme (Egg Regulation[Error! Bookmark not defined.]) commenced on 18 June 2010. Inspections of egg producers started in October 2010 and audits of egg producers/graders started in March 2011.

In 2009, the NSW Food Authority (the Authority) completed a detailed hazard analysis for all types of egg businesses and a food safety risk assessment in developing the Egg Regulation (NSW Food Authority, 2009). The analysis highlighted high risk activities such as sorting, grading, washing and storing eggs. The highest risk activities were identified as pulp collection and further processing (pasteurisation).

Pathogenic organisms (*Salmonella*, *Bacillus cereus, Listeria monocytogenes* and *Staphylococcus aureus*) may contaminate the shell of eggs through environmental and faecal contamination. However, when analysing foodborne illness outbreaks attributed to eggs in Australia, virtually all outbreaks implicated *Salmonella,* with *S.* Typhimurium being the dominant serovar responsible (OzFoodNet, 2008, 2009, 2010).

In 2009, the *Australian Salmonella Reference Centre Annual Report* revealed that *S.* Typhimurium was the most common serovar isolated from eggs submitted to the Centre in 2005 (38%) to 2008 (34%), supplanted by *S.* Livingstone (30%) in 2009. *S.* Typhimurium (19%) and *S.* Ohio (19%) were the second most common serovars isolated in eggs in 2009. The report also indicated that *S.* Typhimurium was the most common serovar found in egg laying flocks for 2007 to 2009 (17% in 2007, 25% in 2008, and 28% in 2009) (IMVS, n.d).

As background for this study, the Authority undertook a review of national and international scientific literature on *Salmonella* prevalence in egg laying environments. The table in Appendix 1 lists key findings for each of the identified studies on estimating the apparent prevalence of *Salmonella* in feed, faecal material, litter and hen drinking water. Unfortunately, Australian publications were limited in number. The literature review found that prevalence of *Salmonella* varied greatly between the studies and offered little guidance as to the expected prevalence of *Salmonella* within the egg laying environment and farm inputs for egg farms in NSW.

Currently the Australian flock is rated as being free from *S.* Enteritidis (SE). This is unlike Europe and other countries where stringent regulatory efforts focus on controlling and eliminating *S.* Enteritidis.

1.1 Objectives

From 1 December 2010 to 30 November 2011, the Authority undertook a baseline evaluation study of egg businesses in NSW following the introduction of the Egg Regulation. The microbiological survey of egg producers (egg farms) in NSW intended to:

1. provide the Authority with an up-to-date summary of egg industry risks by estimating prevalence of *Salmonella* on egg farms in NSW, including the establishment of a microbiological profile of *Salmonella* serovars for comparison with notified cases of human salmonellosis in NSW,
2. establish useful microbiological benchmarks for *Salmonella* in the egg laying environment and farm/shed inputs (eg stock feed and drinking water) and *E. coli* (water only) which can serve as a point of reference for assessing the impact of the Egg Regulation in the future, and
3. collect data which provides insight into the appropriateness of the current Egg Regulation requirements by identifying areas of potential concern at the initial regulatory visit. This can then inform development of industry assistance and regulatory (audit/inspection) assistance that are most useful for businesses.

2. Methodology

Under the Egg Regulation, businesses that produce or grade more than twenty dozen eggs for sale in any week are required to be licensed with the Authority. At the time of the survey, there were a total of 199 licensed egg businesses —74 licensees for egg primary production (egg producers) and 125 licensees for egg primary production with additional activities such as grading and washing (egg producers/graders).

2.1 Proportionate numbers of egg businesses were sampled across all regions

Authority officers collected samples from 49 farms (representing 25% of the total number of egg businesses licensed at the time of the survey). Participation in the survey was voluntary. Every attempt was made to randomly select businesses, but selection processes were influenced somewhat by audit/inspection schedule, laboratory availability and biosecurity considerations.

All regions (with licensed egg businesses) except one were sampled within a 4% variance of the total number of licensed businesses in the area (Appendix 2). The exception, the Northern Rivers region, was due to increased numbers of businesses applying for licences late in the sampling period.

Authority officers collected samples from:

- 20 egg producers, representing 27% (20/74) of the total number of egg primary production businesses licensed at the time of the study, and
- 29 egg producers/graders, representing 23% (29/125) of the number of total egg primary production with additional activities businesses licensed at the time of the study.

The Authority provided all participating farms with a written analysis of their survey results, highlighting areas and possible methods of corrective action where appropriate. In planning the survey, the Authority prepared an action plan for *Salmonella* results. It outlines proposed responses for any *S.* Enteritidis detection (Appendix 3).

2.2 Sample type and survey methodology

Eggs were excluded from the sampling plan due to the large number needed. The rate of *Salmonella* contamination of eggs is highly variable and differs from country to country, site of collection (retail versus on-farm) and the types of eggs sampled (production system, washed, unwashed etc). Based on available Australian scientific literature (Daughtry et al., 2005), a suspected prevalence of 1 in 25,000 eggs (0.004%) informed formal sample size calculations indicating that, at a minimum, 2000 eggs would need to be collected from 70 farms in order to generate statistically significant results. As this exceeded the resources available for this study, the scope of the survey was limited to environmental samples.

The decision to omit egg surface and content samples from the survey plan was consistent with findings in the international scientific literature. Wales et al. (2007) stated that persistent environmental contamination on commercial laying farms is considered to be the predominant problem. They contend that sampling the hen house environment, if carried out properly, has been proven to be a sensitive and cost-effective method of monitoring *Salmonella* carriage and excretion by layer hens. Wales et al. (2007) cited a number of studies that concluded that there is good agreement between the level of environmental contamination and the level of internal egg contamination and associated human disease.

Scientific literature informed selection of sample type. Environmental factors such as humidity and temperature, and management practices such as animal density, housing and stock feed, can influence the *Salmonella* status of a flock (Angen et al., 1996; Opara et al., 1992). As such, farm/shed inputs such as stock feed and drinking water were included in the sampling regime.

A number of studies provide ample justification for including stock feed in the sampling regime. It is recognised that stock feed has the potential to be a common vehicle for bringing *Salmonella* and other pathogens into the farm environment (Jones & Richardson, 2004; Sanchez et al., cited in Jones 2011). Contaminated stock feed may increase the risk of contamination of birds and eggs (external at least) through direct or indirect contact.

Hen drinking water was also included as a potential risk factor for egg contamination. Drinking water may be contaminated with *Salmonella* by way of faecal material, feed and dust deposit in drinkers or by residual contamination in the drinkers (Poppe et al., 1985). Furthermore, Dhillon et al. (1974) demonstrated that contaminated water was a more effective route of transmission of *Salmonella* than stock feed.

The sample schedule was informed by a statistical model[4] *which was used to calculate optimum sample numbers.* For each of the four types of samples, sample number targets were established. For all but one sample type (faecal material), an optimum number of samples was obtained (see Appendix 4). Overall, 99 boot/cage swabs and 90 faecal material samples were collected from the egg laying environment. Farm/shed input samples comprised 128 stock feed and 66 drinking water samples. In total, 383 samples were collected from 49 farms. In the laboratory, all samples were analysed qualitatively for *Salmonella* and some water samples were also tested quantitatively for *E. coli* using the appropriate Australian Standard methods (Appendix 5).

Authority officers used standardised sample collection methods. In this survey, every attempt was made to control the effect of methods on sampling accuracy by using standardised collection methods as outlined in Appendix 5.

Authority officers collected samples from a maximum of four sheds per farm[5]. From each shed, a set of four samples was collected comprising boot/cage swab, faecal material, feed at point of consumption and hen drinking water. For farms with less than four sheds, each shed was sampled as described. If available, samples of bulk stored feed and drinking water source samples were also taken from the main storage silo and water storage reservoir (tank) for each farm.

At most, eighteen samples (including bulk stored feed and drinking water source water source) were collected from each farm. At the time of sampling, Authority officers noted industry profile details including egg farm production system, egg production volume, shed quantity, flock age and seasonality.

Samples were transported to the laboratory under temperature control. All samples were packed in insulated containers with chilled packs and transported to NSW Forensic and Analytical Science Services for testing. Samples were kept under temperature control at all times and all samples, except faecal material, were tested within twenty four hours of sampling[6].

Analysing the results. *Salmonella* prevalence was analysed for egg laying environment and farm/shed inputs. The results distinguish between *Salmonella* serovars and *S.* Typhimurium where relevant. *S.* Typhimurium isolates were sent for further analysis via phage and MLVA[7] typing.

Consistent with the classification system outlined by Sotto, Litiere and Aerts (2011) in their statistical evaluation report of European Union *Salmonella* monitoring data, each farm was classified as 'positive' if *any* sample from that farm was positive for *Salmonella.* A shed was classified as 'positive' if any boot/cage swab, faecal material, feed at point of consumption or hen drinking water sample was positive for *Salmonella*. A flock[8] was classified as 'positive' if any boot/cage swab or faecal material was positive for *Salmonella*. Farms, sheds and flocks were 'negative' if all relevant samples were negative.

Further analysis of the data resulted in *Salmonella* prevalence for a number of subcategories including egg farm production system, egg production volume, flock age, season and region (see Appendices 7–11). However, it is important to note that based on this level of sampling, statistically valid conclusions cannot be made when assigning cause to any observed differences.

[4] Normal approximation of the binomial distribution.
[5] On average, it is assumed that each egg farm houses four sheds. It also takes into account the laboratory capacity.
[6] Faecal samples were frozen for 24 hours before sent to the lab to eliminate live insects and their eggs.
[7] MLVA: multilocus variable number tandem repeat analysis.
[8] In total, 25% of sheds surveyed housed more than one flock (ie flocks of different ages).

2.3 Wet weather conditions prevailed throughout the sampling period but sample days were generally dry

As stated by Opara et al. (1992), *Salmonella* status of a flock can be influenced by environmental parameters, especially humidity. As the sampling collection period spanned over twelve months, it is important to note overall weather trends for future benchmarking purposes.

Generally, weather conditions during the sampling period were warm and wet. The Bureau of Meteorology stated that 2011 was the 12th wettest year on record for NSW. However, sampling records indicated dry weather conditions were reported for over 83% (33/40) of egg farms surveyed, while only 7% (7/40) were sampled under wet conditions[9].

3. Results

The aims of the survey included establishing overall baseline prevalence of *Salmonella* and collecting a microbiological profile of surveyed egg farms in NSW. For studies of this nature, it is not unusual for the existence of multiple risk factors including farm production system, production volumes, location, type of feed, season and flock age to have an effect on *Salmonella* prevalence. Due to the small sample numbers of some of the risk factor categories and issues with dependencies between risk factors, it is impossible to tease out the true effect of one factor over another on the prevalence of *Salmonella* over the sample period. Therefore, the simplest form of statistical analysis was undertaken and results are presented as single variable graphs.

It is also worth noting that other factors including sampling accuracy can also affect prevalence estimations and must be taken into consideration when analysing the findings, even though every effort was made to control this.

3.1. *Salmonella* was not detected in over half of the egg farms in the study

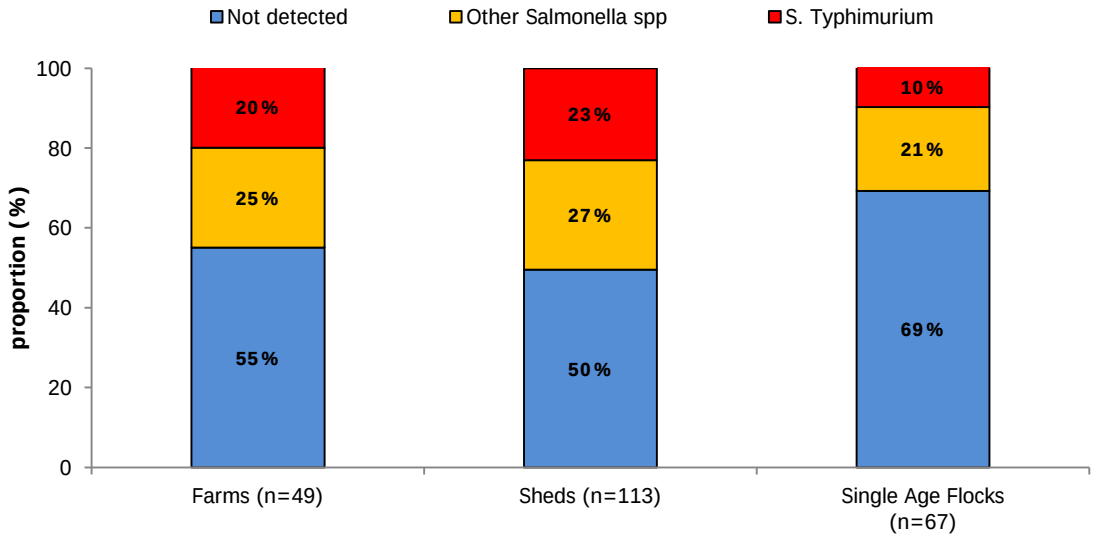
Overall, the survey findings provide insight into the prevalence of *Salmonella* on egg farms at initial regulatory visits. Figure 1 illustrates the prevalence of *Salmonella* on 49 farms, 113 sheds and 166 flocks included in the survey.

Salmonella was detected in 22 of the 49 farms (45%, 95% CI[10] 32-59%). Ten (20%) farms were positive for *S. Typhimurium* and no farm was positive for *S.* Enteritidis. In comparison, prevalence of *S.* Typhimurium was only slightly lower than that identified by the NSW/VIC *Salmonella* Enteritidis (SE) Monitoring and Accreditation Program (Arzey, 2008). Over a period of seven to eight consecutive months for 22 farms in 2006–07 the monitoring program found that *S.* Typhimurium was identified in egg laying environments of almost half (10/22) of the farms assessed. It is worth noting that many of these farms underwent repeated sampling during that time.

At shed level, *Salmonella* was detected in half (56/113) of the sheds sampled. When examined on a per flock[11] basis, 31% (21/67) of single age flocks were positive for *Salmonella* with one-third (7/21) of those flocks positive for *S.* Typhimurium.

[9] Weather conditions were not recorded for nearly 20% (9/49) of businesses included in the survey.
[10] 95% confidence intervals derived from the binomial and beta probability distributions.
[11] Single age flocks only.

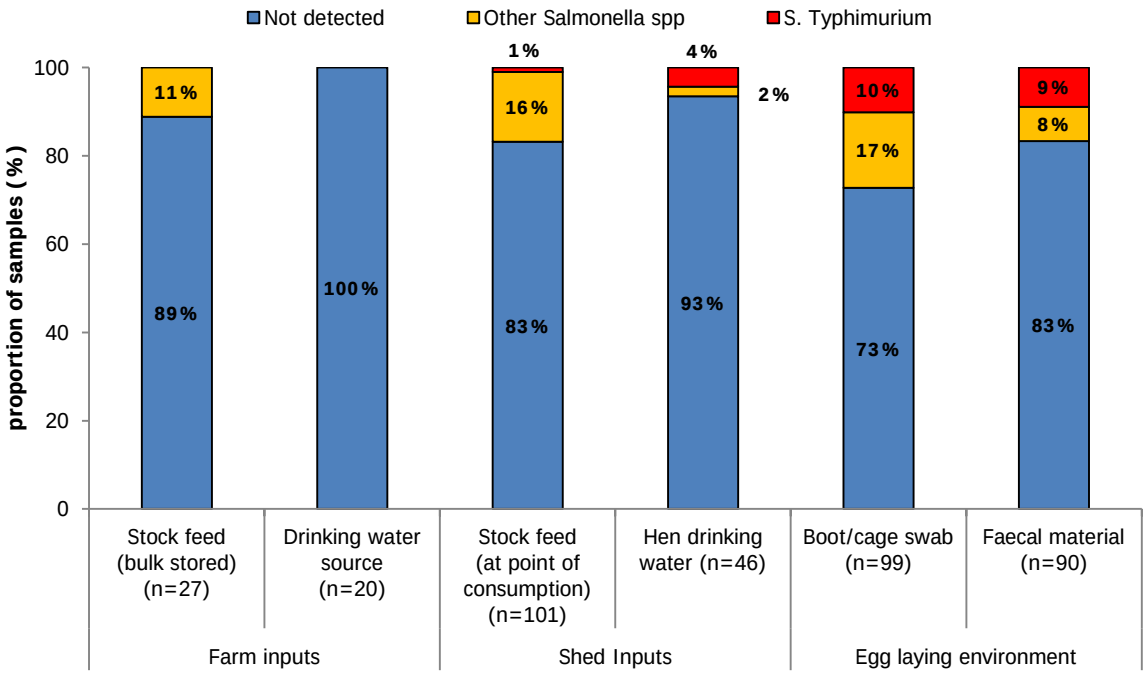
Figure 1. Prevalence of *Salmonella* on farms, sheds and flocks



3.2 Best practice implementation includes benchmarking *Salmonella* prevalence in farm and shed inputs and in the egg laying environment

Figure 2 presents *Salmonella* prevalence for farm/shed inputs and egg laying environment for the farms surveyed. Overall, it shows that egg laying environment samples (boot/cage swabs and faecal material) had higher *Salmonella* prevalence than the shed and farm inputs (stock feed and drinking water samples).

Figure 2. *Salmonella* prevalence in egg laying environment and farm inputs



Farm inputs

In general, *Salmonella* prevalence in farm inputs (bulk stored feed and drinking water source) is relatively low.

Of the farms with bulk stored feed[12], 11% (3/27) of samples tested positive for *Salmonella*, but no sample tested positive for *S.* Typhimurium.

Of the twenty drinking water source samples (reticulated and non-reticulated), none were positive for *Salmonella*. Only ten of the water source samples (nine non-reticulated and one reticulated) were tested for indicators of faecal contamination[13]. Half (5/10) of the samples (all non-reticulated) contained detectable levels of *E. coli*[14]. Results ranged from 1–66 CFU/ml.

Shed inputs

Figure 2 shows *Salmonella* prevalence for shed inputs such as feed at point of consumption and hen drinking water for the surveyed farms—17% (17/101) and 6% (3/46), respectively.

Higher *Salmonella* prevalence in feed at point of consumption (shed input) was expected resulting from increased risk of cross contamination from chicken faeces, dust and floor litter in the shed (Sasipreeyajan et al., 1996). In fact, *Salmonella* prevalence was almost one-third higher for feed at point of consumption (17%, 17/101), compared with levels found in bulk stored feed (11%, 3/27).

The published scientific literature provides little guidance on expected *Salmonella* prevalence in stock feed (Appendix 1). Published prevalence data was extremely variable, ranging from 0.5% (Shirota et al., 2001) to 78% (Cox et al., 2002). Shirota et al. (2001) sampled feed upon arrival, directly from the delivery truck. Conversely, Cox et al (2002) sampled complete feed and feed components on farms in South Eastern Queensland. It is difficult to know whether their samples were of bulk stored feed or collected at point of consumption.

In this survey, only five of the 46 hen drinking water samples were tested for *E. coli* (three from reticulated and two from non-reticulated sources). Three of the five samples (two from reticulated and one from non-reticulated sources) contained detectable levels of *E. coli*, ranging from 2 to 470,000 CFU/ml. Again, due to suspected cross contamination in the sheds, higher levels of *E. coli* were detected in hen drinking water compared with the water source samples.

Egg laying environment

In the egg laying environment, *Salmonella* was detected in just over one-quarter (27/99) of boot/cage swab samples. *Salmonella* prevalence was lower for faecal material (17%, 15/90). These findings are on par with results from previous studies. Van Hoorebeke et al. (2009) explains that bacteriological analysis of faecal material most probably underestimates the actual prevalence of *Salmonella* in laying hen flocks because hens may carry the pathogen without shedding.

For the ten farms positive for *S.* Typhimurium, isolates from 21 samples were subjected to further typing using MLVA. Of these, isolates from seven environmental samples were identical to two STm MLVA types isolated from human cases in NSW in 2011. Further analysis revealed that the isolates from three hen drinking water and feed at point of consumption samples were of different STm MLVA types than those identified in human salmonellosis notifications.

Salmonella serovars in bulk stored feed not always found in the shed

Further analysis of the results highlighted the fact that contaminated bulk stored feed can become an endemic issue in laying flocks when the same *Salmonella* serovar is also detected in the egg laying environment. Data from the three farms with *Salmonella* positive bulk stored feed samples were examined closely. Even though the serovars identified in bulk stored feed were not always identical to those found in the shed, cross contamination is possible from chicken faeces, dust and floor litter in

[12] Bulk stored feed samples and water source samples were not available at all farms.
[13] Water samples were not tested for *E.coli* if the sample arrived at the laboratory after 24 hours of collection.
[14] Limit of detection is 1 CFU/ml.

the shed. These findings are therefore most likely a reflection of low levels/sporadic contamination of the feed and the small sample numbers rather than indicating the absence of cross contamination.

- Farm 1 – *S.* Senftenberg was found in the bulk stored feed and in the feed at point of consumption in three of the four (75%) sheds, and in the faecal matter in half (2/4) of the sheds. A different serovar (*S.* Livingstone) was detected in the feed at point of consumption from the fourth shed. This business produced their feed in-house.
- Farm 2 – *S.* Havana was detected in both the bulk stored feed and in the feed at point of consumption. However, the boot/cage swab sample was positive for two other serovars (*S.* Bredeney and *S.* Orion). This business produced their feed in-house.
- Farm 3 – the bulk stored feed was positive for both *S.* Senftenberg and *S.* Johannesburg. However, the feed at point of consumption was positive for *S.* Singapore. This business purchased its feed from an external feed supplier.

Similar *Salmonella* prevalence for self-produced and purchased feed

The survey reported that one-quarter (12/48) of egg farms in the study produced their own feed. *Salmonella* prevalence in self-produced feed is of interest, as a UK study found that on-site contamination of feed by wildlife appears to be more of an issue with on farm mixers compared with dedicated feed mills (Davies & Wales, 2010). The authors reasoned that the opportunities for rodents and wild birds to live and acquire *Salmonella* from the surrounding environment are usually greater on a farm than at a feed mill.

In order to account for possible cross contamination from *Salmonella* in the egg laying environment, only bulk stored feed samples were further assessed. No apparent difference in *Salmonella* prevalence was observed between purchased and self-produced feed. *Salmonella* was detected in 10% (2/20) of purchased feed samples. In total, 14% (1/7) of self-produced feed samples tested positive for *Salmonella.*

No *Salmonella* prevalence data for dry mash and pellets[15]

Observations regarding differences in *Salmonella* prevalence between dry mash and pelleted feed were not possible from this survey as all bulk stored feed samples were dry mash. As stated earlier, only bulk stored feed was further assessed in order to rule out the effect of cross contamination on *Salmonella* prevalence from the egg laying environment. However, it is worth noting that over 60% (26/42) of the egg farms in the study used dry mash feed compared to 38% (16/42) using pellets. No farm in the study was observed using wet mash.

It is interesting to note that a number of overseas studies found that dry mash was generally more contaminated than pelleted feed samples (Jones et al., 1991; Jones & Richardson, 2004; Veldman et al., 1995). Jones (2011) highlighted the fact that, during the pelleting process, the addition of steam destroys *Salmonella.* However the reduction in *Salmonella* prevalence in pellet varies in accordance with a range of factors including initial levels in the feed ingredients, lethality of the heat treatment and the possibility of cross contamination from the environment during pellet cooling and storage steps (Jones, 2011).

[15] The most common types of feed used in the chicken industry are mash (dry or wet), pellets and crumble (Jahan et al., 2006; Jones, 2011).

- Dry mash is a form of a complete feed that is finely ground and mixed so that birds cannot easily separate out ingredients.
- Wet mash is usually made from mixing dry mash or pellets with hot water.
- Pellet is a form of complete feed that is compacted and extruded. The pelleting process comprises mixing steam with mash feed (conditioning), pressing conditioned feed through metal dies (pelleting) and removing heat and moisture via large volumes of air (cooling).
- Crumble is a type of feed prepared by pelleting the mixed ingredients and crushing the pellet to a consistency coarser than mash.

3.3 Observations on *Salmonella* prevalence by production system, production volume, flock age, season and location

The survey design does not allow for causal inferences to be drawn about the influence of production system, production volume, flock age, season and location on *Salmonella* prevalence. Observations when the data is analysed by these categories is detailed in Appendices 7–11 and summarised below.

Production system

Of the 49 egg farms included in the study, 30 used a free-range system, 15 used a cage-based system (multi-tier or single tier) and four used a barn system. *Salmonella* was detected across all production systems. *Salmonella* prevalence for both cage-based and free-range systems was comparable, ie 40% (6/15 and 12/30 respectively). Multi-tier cage and barn systems had the highest *Salmonella* prevalence, and single-tier cage systems had the lowest.

Production volume

The production volume from farms included in the survey ranged from <1000 eggs per day up to 250,000 eggs per day. *Salmonella* was detected on farms regardless of production volume. A higher prevalence of *Salmonella* was found on farms producing greater volumes of eggs. *S.* Typhimurium was also detected in egg farms regardless of their production volumes.

Flock age

Flocks on farms in the study ranged from 21 to 80 weeks in age. In total, there were 67 sheds housing single age flocks. *Salmonella* prevalence was determined for eight flock age categories. *Salmonella* was detected across flocks of all ages but prevalence was highest for the youngest flock age categories (< 20 weeks and 21-30 weeks). No obvious signs of confounding due to flock age were identified.

Season

The sampling period for the study was twelve months. Samples were collected from farms from December 2010 to November 2011. In all but two months of the sampling period, *Salmonella* was detected on farms (April and November 2011). Overall, a higher prevalence of *Salmonella* was found during the summer months (31%, 24/77). *Salmonella* prevalence during spring was the lowest (8%, 9/98).

Location

NSW is divided into twelve regional areas. As no farms are located in Far West NSW, surveyed farms were located in all but one of the regional areas. *S*. Typhimurium was detected on farms in six of the eleven regions. However, because the number of farms in some regions was small it is impossible to make any inferences about location and the prevalence of *S.* Typhimurium.

Survey findings provide a solid evidence base for informing the design of surveys in the future. There are opportunities for further work examining the influence of these categories on *Salmonella* prevalence when assessing the impact of the Egg Regulation in the future.

3.4 Benchmarking *Salmonella* prevalence on egg farms is one measure of regulatory effectiveness

The number of farms with positive *Salmonella* samples ('positive farms') and the proportion of positive samples on those farms provide an overall benchmark for monitoring the impact of the Regulation in the future. The proportion of positive samples provides a general indication of the overall effectiveness of food safety management practices. For over half (27/49) of the farms, *Salmonella* was not isolated from any sample.

As presented in Table 1, selected percentile rankings for 'positive farms' were calculated as follows: For half of the *Salmonella* positive farms (at the 50th percentile, at most, the proportion of positive *Salmonella* samples was 27%. For 95% of the *Salmonella* positive farms (at the 95th percentile), at most, two-thirds of the samples were positive. For 99% of positive farms (at the 99th percentile), at most, 93% of the samples were positive. It is worth noting that for each farm the number of samples varied in accordance with the number of sheds. For farms with at least four sheds, bulk

stored feed and tanked drinking water, at most, 18 samples were collected. For a farm with one shed, with no bulk stored feed or tanked water, only four samples were collected.

This data provides a benchmark against which to compare the impact of the Regulation over time. Ideally, future trends would show decreasing *Salmonella* prevalence on farms with fewer positive sites on farms. This would be seen as an overall downward shift in the proportion of positive samples per farm across all percentile rank categories. However, due to the fact the reliability of this approach is untested, the Authority would consider measures in addition to *Salmonella* prevalence on farms.

Table 1. Egg farms in this study ranked by proportion of *Salmonella* positive samples

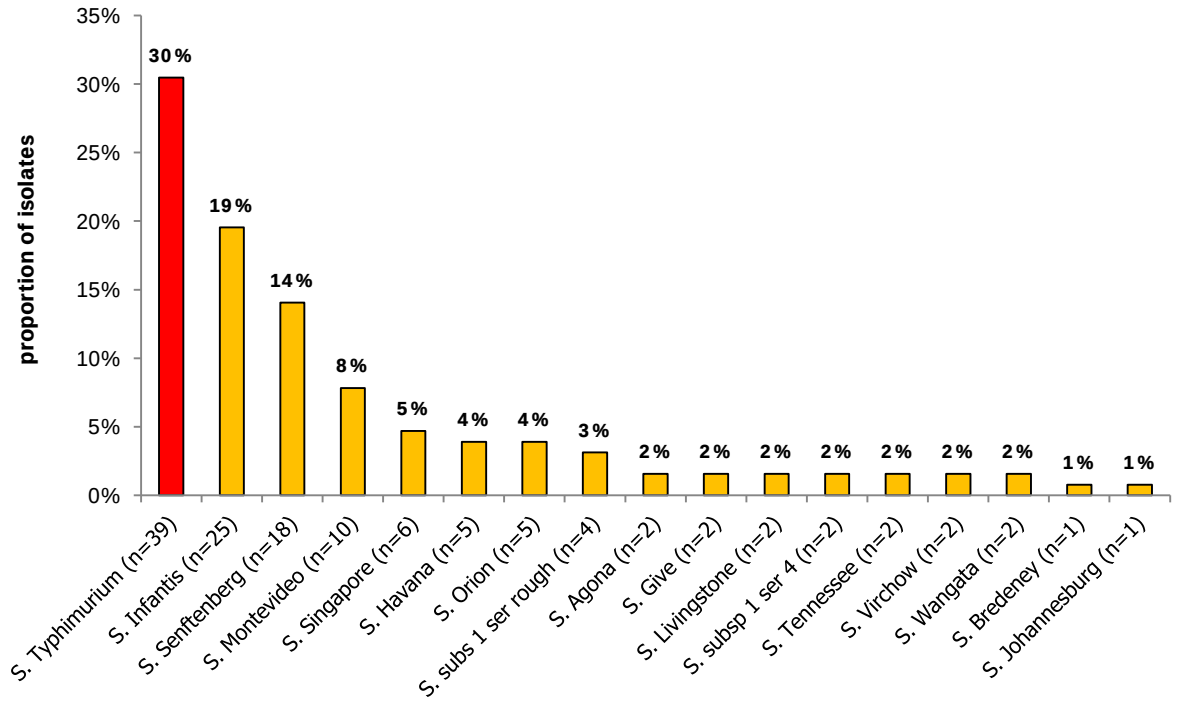
Percentile ranking	% of positive samples
Median[16]	27%
90th percentile	60%
95th percentile	66%
99th percentile	93%

3.5 *S.* Typhimurium was the most frequently occurring serovar

Figure 3 presents a profile of *Salmonella* serovars among the isolates tested. For each positive sample (n=65), two isolates were selected for serovar analysis, resulting in a total of 130 isolates. With the exception of five samples, isolate pairs were the same for each sample.

In total, seventeen serovars were isolated across the *Salmonella* positive egg farms in the survey. *S.* Typhimurium predominated (30%, 39/130) on egg farms in the survey, followed by *S.* Infantis (19%, 25/130), *S.* Senftenberg (14%, 18/130) and *S.* Montevideo (8%, 10/130). *S.* Enteritidis was not detected in any sample.

Figure 3. *Salmonella* serovars isolated in this study



[16] Number of samples taken per farm ranged from 4 to 18 depending on the number of sheds. Summary statistics were calculated for the 22 farms with *Salmonella* positive samples.

It appears that changes may have occurred in the serovar profile on egg farms in NSW over time. The joint NSW/VIC *S*. Enteritidis Monitoring and Accreditation Program data for egg farms was analysed and serovar profiles were established for distinct periods of time (Arzey, 2008).

From 1996 to 2000, monitoring results indicated *S*. Sofia was the most prominent serovar followed by *S*. Agona. From 2001 to 2003, it was *S.* Agona followed by *S.* Infantis. From 2004 to 2005, *S.* Mbandaka and *S.* Typhimurium were the two most dominant serovars detected by the monitoring program (Arzey, 2008).

However, it is uncertain how these survey findings compare with other Australian jurisdictions, as published research on *Salmonella* serovars in Australian poultry related environments is limited. Chinivasagam et al. (2010) collected samples from 28 broiler farms in Queensland (n=10), NSW (n=9), and Victoria (n=9) and found *S*. Sofia (70%) to be the dominant serovar, followed by *S*. Virchow (10%) and *S*. Chester (10%).

A survey of four selected egg farms located in South East Queensland found that *S*. Singapore (23%) was the dominant serovar in both feed and faecal material samples (Cox et al., 2002).

3.6 Egg farms and human notifications in NSW – two *Salmonella* serovars common to both

Salmonellosis is a notifiable disease in NSW, meaning laboratories are required by law to notify Public Health Units of every positive human stool culture. Notifications of human salmonellosis are characterised according to specific serovars of *Salmonella*. *S.* Typhimurium was further characterised by phage and MLVA typing.

Overall, in 2011, *S.* Typhimurium accounted for 57% of human notifications in NSW (J. Musto, personal communication, August 2012).

Table 2 lists the top ten *Salmonella* serovars most frequently identified in this survey and those isolated in humans (listed in order of notification frequency). Two common serovars were identified, *S.* Typhimurium and *S.* Infantis.

Table 2. Top ten *Salmonella* serovars isolated on egg farms in this study and in humans in NSW

	Egg farms (2011)		Humans (2011)	
	Salmonella serovars	Prevalence	*Salmonella* serovars[17]	No. of notifications (%)
1	**Typhimurium**	39 (30%)	**Typhimurium**	1972 (57%)
2	**Infantis**	25 (20%)	Enteritidis[18]	174 (5%)
3	Senftenberg	18 (14%)	Virchow	159 (4.6%)
4	Montevideo	10 (8%)	Wangata[19]	89 (2.6%)
5	Singapore	6 (5%)	**Infantis**	74 (2.1%)
6	Havana	5 (4%)	Paratyphi B by Java	72 (2%)
7	Orion	5 (4%)	Birkenhead	71 (2%)
8	Subs 1 ser rough	4 (3%)	Saintpaul	50 (1.4%)
9	Agona	2 (2%)	Bovismorbificans	44 (1.3%)
10	Give	2 (2%)	Newport	38 (1.1%)

[17] These *Salmonella* serovars caused illness in humans.
[18] *S*. Enteritidis is mostly overseas acquired (87%).
[19] S. Wangata, *S*. Paratyphi B by Java and *S*. Birkenhead are environmental serovars.

3.7 Egg farms and human notifications in NSW – similar *S.* Typhimurium phage type profiles

Table 3 lists the top five phage types identified in the egg farm survey and from human salmonellosis notifications in NSW (J. Musto, personal communication, November 2012). It shows that four of the top five *S.* Typhimurium phage types are common to both egg farms and human notifications.

In total, six *S.* Typhimurium phage types were isolated from the surveyed egg farms in NSW. The analysis found that the most frequently isolated phage type was STm 170 (also known as STm 108), accounting for 43% of all positive samples. This phage type was also most frequently isolated in humans in 2011 and egg layers in 2009 (IMVS 2009 Annual Report, n.d.).

Table 3. Most frequently isolated *S.* Typhimurium phage types on egg farms and human notifications in NSW

	Egg farms (2011)		Egg layers (2009)[20]	Humans (2011)	
	Phage types	Prevalence (n=21)	(n=121)	(likely) Phage types	No. of notifications (%)
1	170/108	9 (43%)	63 (52%)	170	684 (20%)
2	9	3 (14%)	12 (10%)	44	172 (5%)
3	135	3 (14%)	6 (5%)	9	156 (4%)
4	197	3 (14%)	--	135a	149 (4%)
5	135a	2 (10%)	--	135	134 (4%)
6	8	1 (5%)			

3.8 MLVA typing – two types common to egg farms and human notifications in NSW

MLVA stands for multilocus variable number tandem repeat analysis, which is a typing method used to differentiate strains of *S.* Typhimurium. MLVA typing is more precise than phage typing and enables DNA 'fingerprinting' of *S.* Typhimurium isolates. More information on the MLVA typing process has been included in Appendix 6.

S. Typhimurium MLVA 3-9-7-13-523 was the most frequently detected MLVA type both in this study and isolated in humans in NSW in 2011. *S.* Typhimurium MLVA 3-9-7-15-523 was the only other MLVA type in common (Table 4).

[20] As reported in IMVS 2009 Annual report

Table 4. Top ten ranked *S*. Typhimurium MLVA types isolated on egg farms in this study and humans in NSW

	Egg farms (2011)		Humans (2011)	
	MLVA	Prevalence	MLVA	No. of notifications (%)
1	**3-9-7-13-523**	41% (7/17)	**3-9-7-13-523**	249 (7%)
2	5-13-11-9-490	18% (3/17)	3-10-8-9-523	147 (4%)
3	**3-9-7-15-523**	12% (2/17)	3-9-8-13-523	112 (3%)
4	3-23-14-13-523	12% (2/17)	3-9-7-14-523	90 (3%)
5	3-23-11-13-523	6% (1/17)	3-12-9-10-550	75 (2%)
6	3-12-10-13-523	6% (1/17)	**3-9-7-15-523**	59 (2%)
7	3-10-16-12-496	6% (1/17)	3-14-11-12-523	49 (1%)
8	--	--	3-10-14-12-496	46 (1%)
9	--	--	3-12-15-13-523	46 (1%)
10	--	--	3-13-11-9-523	29 (<1%)

4. Conclusion

The ultimate aim of the Egg Regulation is to reduce the incidence and potential for foodborne illness from eggs and egg products. This survey findings confirm the importance of the regulatory food safety measures for the egg industry in NSW as *Salmonella* was detected on close to half the farms surveyed. Many of the *Salmonella* types that predominated on farms also predominate among isolates from humans. While there is sufficient evidence in the scientific literature to show that the presence of *Salmonella* in the egg laying environment does not automatically infer a high prevalence on whole eggs offered for sale, it does highlight increased risk associated with cross contamination of *Salmonella* from the environment to whole eggs.

As best practice implementation, survey data was used to benchmark *Salmonella* prevalence in egg laying environments and farm inputs, and *E. coli* (water only) for farms in NSW. Data provides a point of comparison for assessing future impacts of the Egg Regulation and for monitoring any changes to composition and activities of the NSW egg industry.

The survey findings have also provided insight into the appropriateness of Egg Regulation requirements by highlighting areas of potential concern at the initial regulatory visit. In future, the Authority plans to work with industry to ensure that risk management approaches remain targeted and effective, especially in relation to reducing:

- flock-to-flock transmission of *Salmonella*,
- contamination risks for feed and feed ingredients, and
- potential for contamination of drinking water (water source and hen drinking water).

Due to the lack of published scientific literature for Australian egg farms, the survey data also provides a solid evidence base upon which to build future findings. However, when evaluating the impact of the Egg Regulation in the future, as a matter of practice, the Authority will consider other measures in addition to the apparent prevalence of *Salmonella* on farms. Other strategies for examining the causal impacts of the Regulation are to be included, as it is usual for microbiological surveys of this kind to be subject to a number of confounding factors such as the effect of time, seasonality and flock age.

References

Angen, Ø., Skov, M.N., Chriél, M., Agger, J.F. & Bisgaard, M. (1996). A retrospective study on salmonella infection in Danish broiler flocks. *Preventive Veterinary Medicine, 26*, 223 – 237

Arzey, G.G. (2008) Persistence of *Salmonella* on layers farms and implications to foodborne risks and risk interventions. Paper published in the Proceedings of the WPSA, Brisbane.

Bureau of Meteorology. (2011). *NSW in 2011: Wet, warm year as La Niña ends and another begins*. Retrieved 2 March 2012 from http://www.bom.gov.au/climate/current/annual/nsw/summary.shtml.

Carrique-Mas, J.J., Breslin, M., Snow, L., McLaren, I., Sayers, A.R., Davies, R.H. (2009). Persistence and clearance of different *Salmonella* serovars in building housing laying hens. *Epidemiology and Infection, 137*, 837 – 846

Chinivasagam, H.N., Redding, M., Runge, G. & Blackall, P.J. (2010). Presence and incidence of food-borne pathogens in Australian chicken litter. *British Poultry Science, 51*(3), 311 – 318

Cox, J., Woolcock, J. & Sartor, A. (2002). *The significance of Salmonella, particularly S. Infantis, to the Australian egg industry*. A report of the Rural Industries and Development Corporation.

Daughtry, B., Sumner, J., Hooper, G., Thomas, C., Grimes, T., Horn, R., et al. (2005). *National food safety risk profile of eggs and egg products. Part 1: Final report Part 2: Attachments.* Final report for the Australian Egg Corporation Limited. AECL Publication No 05/06 AECL Project SAR-47

Davies, R. & Breslin, M. (2004). Observations on *Salmonella* contamination of eggs from infected commercial laying flocks where vaccination for *Salmonella* enterica serovar Enteritidis had been used. *Avian Pathology, 33*(2), 133 – 144

Davies, R.H. & Wales, A.D. (2010). Investigations into *Salmonella* contamination in poultry feedmills in the United Kingdom. *Journal of Applied Microbiology, 109*, 1430 – 1440

Dawson, R.C., Cox, J.M., Almond, A. & Moses, A. (2001). *Food Safety Risk Management in Different Egg Production Systems*. A report for the Rural Industries Research and Development Corporation. RIRDC Publication No 01/111 RIRDC Project No MS989-28.

Dhillon, A. S., Maurer, A. J., Deibel, R. H. & Haller, R. W. (1974). Feeding of different levels of *Salmonella* to Chickens. *Indian Journal of Poultry Science, 9*, 103

FSANZ (2009). *Proposal P301 Primary Production and Processing Standard for Eggs and Egg Products Risk Assessment of Eggs and Egg Products*. Retrieved September 17, 2010 from http://www.foodstandards.gov.au/_srcfiles/P301%20Eggs%20PPPS%20DAR%20SD1%20Risk%20Assessment.pdf

Higgins, R., Malo, R., René-Roberge, E. & Gauthier, R. (1982). Studies on the dissemination of *Salmonella* in nine broiler-chicken flocks. *Avian Diseases, 26*(1), 26 – 33

Holt, P.S., Davies, R.H., Dewulf, J., Gast, R.K., Huwe, J.K., Jones, D.R. et al. (2011). *Emerging issues: social sustainability of egg production symposium. The impact of different housing systems on egg safety and quality*. Presented at the joint annual meeting of the Poultry Science Association, American Society of Animal Science, and American Dairy Science Association, Denver, Colorado 11 – 15 July 2010

IMVS. (n.d). 2009 *Annual report – Australian Salmonella reference centre*. Adelaide, SA.: IMVS.

Irwin, R.J., Poppe, C., Messier, S., Finley, G., & Oggel, J. (1994). A national survey to estimate the prevalence of *Salmonella* species among Canadian registered commercial turkey flocks. *Canadian Journal of Veterinary Research, 58*, 263 – 267

Jahan, M.S., Asaduzzaman, M., & Sarkar, A.K. (2006). Performance of broiler fed on mash, pellet and crumble. *International Journal of Poultry Science, 5*(3), 265 – 270.

Jones, F., Axtell, R., Rives, D., Scheideler, S., Tarver, F., Walker, R. et al. (1991). A survey of *Salmonella* contamination in modern broiler production. *Journal of Food Protection, 54*, 502 – 507

Jones, F.T. (2011). A review of practical *Salmonella* control measures in animal feed. *Journal of Applied Poultry Research, 20*, 102 – 113

Jones, F.T., Axtell, R.C., Rives, D.V., Scheideler, S.E., Tarver JR, F.R., Walker, R.L. et al. (1991). A survey of *Salmonella* contamination in modern broiler production. *Journal of Food Protection, 54*(7), 502 – 507

Li, X., Payne, J.B., Santos, F.B., Levine, J.F., Anderson, K.E. & Sheldon, B.W., (2007). *Salmonella* populations and prevalence in layer faeces from commercial high-rise houses and characterization of the Salmonella isolates by serotyping, antibiotic resistance analysis, and pulsed field gel electrophoresis. *Poultry Science, 86*, 591 – 597

Lindstedt, B.A., Vardund, T., Aas, L., Kapperud, G. (2004). Multiple-locus variable-number tandem-repeats analysis of *Salmonella* enterica subsp. enterica serovar Typhimurium using PCR multiplexing and multicolor capillary electrophoresis. *Journal of Microbiological Methods, 59*(2), 163 – 72

Linton, A.H., Al-Chalaby, Z.A. & Hinton, M.H., (1985). Natural subclinical *Salmonella* infection in chickens: a potential model for testing the effects of various procedures on *Salmonella* shedding. *Veterinary Record, 116* (14), 361 – 364

McChesney, D., Kaplan, G. & Gardner, P. (1995). FDA survey determines *Salmonella* confirmation.

Feedstuffs, 20 – 23

Miles, D. and Chan, C. (unpublished). *Risk Profile and Risk Management of Eggs and Egg Products in NSW*. Internal NSW Food Authority report.

Namata, H., Méroc, E., Aerts, M., Faes, C., Abrahantes, J.C., Imberechts, H., & Mintiens, K. (2008). *Salmonella* in Belgian laying hens: an identification of risk factors. *Preventive Veterinary Medicine, 83*, 323 – 336

NSW DPI (unpublished) *Guidelines- Joint NSW/Victoria Salmonella Enteritidis Monitoring and Accreditation Program*. NSW Primary Industries Division of Industry and Investment Department. (J-NSW/VIC SEMAP).

NSW Food Authority. (2009). *Food Safety Risk Assessment of NSW Food Safety Schemes*. Retrieved July 25, 2012 from http://www.foodauthority.nsw.gov.au/_Documents/science/Food_Safety_Scheme_Risk_Assessment.pdf

NSW Food Authority (2012). *Baseline evaluation of the NSW Egg Food Safety Scheme: Survey of NSW egg businesses – industry profile and observed practices*. Retrieved from www.foodauthority.nsw.gov.au

Olsen, A. R. & Hammack, T.S. (2000). Isolation of *Salmonella* from the housefly, *Musca domestica* L., and the dump fly, *Hydrotaea aenescens* (Wiedemann) (Diptera:Muscidae), at caged-layer houses. *Journal of Food Protection, 63*(7), 958 – 960

Opara, O.O., Carr, L.E., Russek-Cohen, E., Tate, C.R., Mallinson, E.T., Miller, R.G. et al. (1992). Correlation of water activity and other environmental conditions with repeated detection of *Salmonella* contamination on poultry farms. *Avian Diseases, 36*, 664 – 671

OzFoodNet. (2008). *Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: annual report of the OzFoodNet Network, 2007*. Retrieved August 21, 2012 from http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3204-pdf-cnt.htm/$FILE/cdi3204c.pdf

OzFoodNet. (2009). *Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: annual report of the OzFoodNet Network, 2008*. Retrieved August 21, 2012 from http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3304-pdf-cnt.htm/$FILE/cdi3304c.pdf

OzFoodNet. (2010). *Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: annual report of the OzFoodNet Network, 2009*. Retrieved August 21, 2012 from http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3404-pdf-cnt.htm/$FILE/cdi3404b.pdf

Poppe, C., Irwin, R., Forsberg, C., Clarke, R. & Oggel, J. (1991). The prevalence of *Salmonella* Enteritidis and other *Salmonella* among Canadian registered commercial layer flocks. *Epidemiology & Infection, 106*, 259 – 270

Poppe, C., Barnum D.A., & Mitchell, W.R. (1985). Effect of chlorination of drinking water on experimental *Salmonella* infection in poultry. *Avian Diseases, 30*, 362 – 369

Renwick, S.A., Irwin, R.J., Clarke, R.C., McNab, W.B., Poppe, C., McEwen, S.A. (1992). Epidemiological associations between characteristics of registered broiler chicken flocks in Canada and the *Salmonella* culture status of floor litter and drinking water. *Canadian Veterinary Journal, 33*, 449 – 458

Sasipreeyajan, J., Jerngklinchan, J., Koowatananukul, C., & Saitanu., K. (1996). Prevalence of *Salmonella*e in broiler, layer and breeder flocks in Thailand. *Tropical Animal Health Production*, *28*, 174 – 180

Sergeant, E., Grimes, T., Jackson, C., Baldock, F. & Whan, I. (2003). *Salmonella Enteritidis surveillance and response options for the Australian egg industry*. A report for the rural industries Research and Development Corporation. RIRDC Publication No 03/006 RIRDC Project No AUV-14.

Shirota, K., Katoh, H., Murase, T., Ito, T. & Otsuki, K. (2001). Monitoring of layer feed and eggs for *Salmonella* in eastern Japan between 1993 and 1998. *Journal of Food Protection, 64*(5), 734 – 737

Sotto C., Litiere, S., Aerts, M., (2011) *Scientific Report Statistical Evaluation of the Achievements by Member States of the EU Salmonella Reduction Targets in Animal Population.* I-Biostat. Available at: http://www.magrama.gob.es/es/ganaderia/legislacion/stat_eval_of_the_achiev_by_memb_stat__eu_tcm7-185560.pdf

Thomas, C., Daughtry, J., James, B., Padula, D., Jordan, J., Davey, G., et al. (2006). *An Egg: Salmonella Quantitative Risk Assessment Model for the Australian Egg Industry*. A Technical Report published by the Australian Egg Corporation Ltd.

United Kingdom Department for Environment, Food and Rural Affairs (DEFRA). (2008). *A Guide to the National Control Programme for Salmonella in laying flocks*. Accessed 13 September 2010 from: http://www.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/zoonoses/documents/reports/ncp-salmonella.pdf.

USDA/APHIS. (2000). *NAHMS Layers '99 Salmonella enterica serotype Enteritidis in table egg layers in the US*. Retrieved August 20, 2012 from http://www.aphis.usda.gov/animal_health/nahms/poultry/downloads/layers99/Layers99_dr_Salmonella.pdf

Van Hoorebeke, S., Van Immerseel, F., De Vylder, J., Ducatelle, R., Haesebrouck, F., Pasmans, F. et al. (2009). Faecal sampling underestimates the actual prevalence of *Salmonella* in laying hen flocks. *Zoonoses Public Health, 56*, 471 – 476

Veldman, A., Vahl, H.A., Borggreve, G.J. & Fuller, D.C. (1995). A survey of the incidence of *Salmonella* species and Enterobacteriaceae in poultry feeds and feed components. *Veterinary Record, 136*(7), 169 – 172

Wales, A., Breslin, M., Carter, B., Sayers, R. & Davies, R. (2007). A longitudinal study of environmental *Salmonella* contamination in caged and free-range layer flocks. *Avian Pathology, 36*(3), 187 – 197

Appendix 1. Selected studies on *Salmonella* prevalence

Published data on *Salmonella* prevalence in farm environment and inputs

Year	Country	*Salmonella* prevalence	Details	Reference
Feed				
2009	Australia	3.5 – 25.6%	No other information available. Personal Communication provided to FSANZ from WA as part of the Egg Primary Production and Processing Standard development process.	FSANZ, 2009
1996	Thailand	8% (n=434)	Feed from 35 flocks of layers, breeders, and broilers	Sasipreeyajan et al., 1996
1995	Australia, QLD	78% (n=63)	Complete feed and feed component samples collected from four layer farms in south east QLD from Sept 1993 – January 1995. Sample collection point unclear.	Cox et al., 2002
1995	USA	56% (n=101) 36% (n=50)	Animal & vegetable protein based feed	McChesney et al., 1995
1991	Canada	7.1% (n=300)	1 kg sample taken from each flock	Poppe et al., 1991
1991	?	35% 6.5%	Unprocessed feeds Processed feeds	Jones et al., 1991
1968	?	0% - 1.6%	Heat-treated Ingredients	Zindel and Bennet in Dawson et al., 2001
1993–1998	Japan	0.5% (n=10,418)	Layer feed sampled upon delivery, before dispensing into bulk storage hopper	Shirota et al., 2001
Faecal material				
1995	Australia, QLD	26% (n=475)	Faecal samples taken from laying sheds of four farms in south east QLD from Sept 1993 – January 1995	Cox et al., 2002
1991	Canada	10.1% (n=5,897)	Faecal	Poppe et al., 1991
1991	Canada	25.7% (n=1,176)	Egg belt	Poppe et al., 1991
Cage/boot swabs				
1996	Thailand	42% (n=85)	Samples taken across 35 farms	Sasispreeyajan et al., 1996
1994	Canada	86.7%	For each flock, 12 pooled litter and 4 dust samples were taken from 270 flocks. (234/270 flocks)	Irwin et al., 1994
2001–2002	Australia	3.1% (95% CI 3.9-2.4%)	n=2,252 samples	Thomas et al., 2006
Hen drinking water				
1996	Thailand	36% (n=89)	Collected from the trough in the bird house	Sasispreeyajan et al., 1996
1996	Thailand	14% (n=42)	Collected from the drinking water source (main tank)	Sasispreeyajan et al., 1996

Appendix 2. Evidence of proportional sampling

The total number of licence holders for egg producers and egg producers/graders was extracted from the Authority's license database and was updated regularly during the period of the study. All licensees were then assigned to one of sixteen regional areas in NSW based on their location. A stratified sampling plan was employed where proportionate numbers of egg businesses per region were randomly selected for sampling.

Proportion of samples taken from total licensees by region

Region	# facilities	Target % of total facilities (n=199)	Actual % of total facilities sampled (n=49)	Actual numbers sampled
Central Sydney	0	0	0	0
Northern Sydney	2	1	0	0
South Eastern Sydney	0	0	0	0
South Western Sydney	29	15	18	9
Western Sydney	29	15	18	9
Central West	16	8	4	2
Far West	1	1	0	0
Hunter	39	20	18	9
Illawarra	3	2	4	2
Mid North Coast	15	8	6	3
Murray	4	2	4	2
Murrumbidgee	7	4	4	2
North West	5	3	2	1
Northern	13	7	8	4
Northern Rivers	19	10	2	1
South East	17	9	10	5
Total	**199**			**49**

Appendix 3. Action plan for *Salmonella* positive results

At the planning stage, the Authority and the NSW Department of Primary Industries (NSW DPI) came up with an agreed action plan for *Salmonella* positive samples (tabled below).

Findings	Action plan	Actioned by
S. Enteritidis isolated in the environmental samples	• The Authority will advise the NSW DPI of the results • A response group will be formed, led by NSW DPI, includes of a representative from the Authority and, potentially, NSW Health	
	• Eggs may be recalled, re-labelled or diverted to pasteurisation	The Authority
	• Further testing[21] (including flock testing)	NSW DPI
S. Enteritidis isolated in a flock	• Quarantine, enhanced biosecurity, vaccination, investigate possible sources and spread, implement preventative measures or additional analytical testing	NSW DPI
S. Typhimurium isolated in any of the samples	• Inform the business in writing of the results, advising them on the appropriate corrective action • Re-visit or re-sample (case-by-case basis)	The Authority

Appendix 4. Sample numbers

Using a statistical model, sample numbers for each sample category were informed by *Salmonella* prevalence as identified by the scientific literature (Appendix 1). Resources, laboratory capacity and biosecurity measures were taken into account when determining a sample number that would provide an acceptable level of statistical reliability.

Sample size calculations were based on the normal approximation of the binomial distribution. Using a statistical model, it was determined that 104 stock feed and faecal matter samples, more than 36 boot/cage swabs, and 111 hen drinking water samples would provide at least 95% confidence of the study estimate being within 5% of the true prevalence—assuming the true prevalence of *Salmonella* on NSW egg farms is $\leq$26% (feed and faecal matter), $\leq$3% (boot/cage swab) and $\leq$36% (hen drinking water).

[21] In accordance with the *Guidelines – Joint NSW/Victoria Salmonella* Enteritidis *Monitoring and Accreditation Program* (Arzey, 2005) and the Rural Industries Research and Development Corporation's (RIRDC) report on *Salmonella Enteritidis surveillance and response options for the Australian Egg Industry* (Sergeant *et al.*, 2003),

The number of samples tested for *Salmonella* in this survey

Sample type	Numbers of samples
Farms	Total 49
• Egg producers	20
• Egg producers/graders	29
Sheds	113
Flocks	166
Egg laying environment	
Faecal material	90
Boot/cage swabs	99
Farm inputs	
• Feed (bulk stored feed)*	27
Shed input	
• Feed (at point of consumption)	101
Hen drinking water	Total 66
• At source (tank)*	20
Reticulated	1
Non-reticulated	19
• At dripper	46
Reticulated	28
Non-reticulated	18

*Bulk stored feed and drinking water source samples were not available from all farms

The number of samples tested for *E. coli* in this survey

Sample type	Numbers of samples
Hen drinking water	Total 15
• At source (tank)	10
Reticulated	0
Non-reticulated	10
• At dripper	5
Reticulated	3
Non-reticulated	2

Appendix 5. Sampling methodology and laboratory methods

Samples	Microorganisms to be tested	Sampling methodology	Australian Standard Method
Feed at point of consumption	*Salmonella*	Approximately 500g of sample were collected from four different areas in each shed	AS 1766.2.5
Bulk stored feed – collected from hopper or silo	*Salmonella*	Approximately 500g of feed was collected per farm	AS 1766.2.5
Boot/cage swab	*Salmonella*	One pair of boot overshoes from each shed Every effort was made when sampling to ensure that boot swabs represented the whole area to which the bird access area. This included all the separate pens, littered and slatted areas (when they are safe to walk on). Overshoes were worn on feet or on hands (cage) and involves taking at least 100 paces to cover an area of approximately 50% of the house (DEFRA, 2008)	AS 1766.2.5
Faecal matter[22]	*Salmonella*	Approximately 200g sample was collected from five different areas in each shed (under cages or under the floor). Samples were frozen for 24 hours before analysis. For multi-tiered cage system, samples were collected from surface and at the end of the faeces belt	AS 5013.10 and AS 1766.2.5
Hen drinking water – collected at point of consumption	*Salmonella*	Approximately 500ml was collected from each farm	AS 4276.14:1995 (modified)
Water source – primary source used for hen drinking or processing[23]	*Salmonella*	Approximately 500ml was collected from the farm's main storage tank	AS 4276.14:1995 (modified)
	E. coli		AS 4276.7:2007

[22] Samples were kept frozen for 24 hours prior to testing.
[23] The NSW Egg Regulation requires that non-reticulated water used for processing (eg washing eggs) is tested for *E. coli* and found not detected in 100mL. For treated water the frequency is six-monthly and for non-treated water it is monthly.

Appendix 6. MLVA purpose and typing process

MLVA is a typing method used to differentiate strains of *Salmonella* Typhimurium for investigation of foodborne outbreaks to identify the contamination source. The purpose of MLVA reporting is to provide genetic analysis to NSW Health and the Authority of the relatedness of *Salmonella* isolates collected from human, food and other sources.

MLVA typing is more precise than phage typing of *S.* Typhimurium and enables DNA 'fingerprinting' of human, food and environmental isolates. This enables greater identification of linkages between human cases involved in foodborne outbreaks.

Summary of the typing process

MLVA typing method involves detection of five repeat gene regions presented on *S.* Typhimurium (*S*Tm) genome. The lengths of the regions vary between each other at the five regions and between isolates, if the isolates are from different sources. To determine the lengths of the regions for each isolate, a polymerase chain reaction (PCR) technique is used to reproduce millions of copies of each of the five repeat gene regions so that they can be accurately measured following separation by capillary electrophoresis. Capillary electrophoresis separates lengths of DNA according to their different sizes by movement through a gel matrix under the influence of an electric current. MLVA type was determined by converting the sizes into codes.

Reliability of the process

The method was initially developed in 2004 by Lindstedt et al. In Demark, this method has been used as part of the national laboratory-based surveillance system of human enteric infections. In Australia, a national MLVA typing network has been established since 2006 with involvement of five major reference laboratories from each state—NSW, Queensland, Victoria, South Australia and Western Australia. As a member of this collaboration network, CIDM-Public Health in NSW applied this method for the typing of *S*Tm based on the agreement and protocol set up by the network.

MLVA results

	Egg laying environment		**Farm-level input**	**Shed-level inputs**		
	Boot/cage swabs	**Faecal material**	**Bulk stored feed**	**Feed at point of consumption**	**Non-reticulated hen drinking water**	**Reticulated hen drinking water**
3-9-7-13-523 (pt 108)	3	4				
3-9-7-15-523 (pt 108)	1	1				
3-10-16-12-496 (pt 9)	1					
3-23-14-13-523 (pt 9)	1				1	
3-12-10-13-523 (pt 35a)		1				
3-23-11-13-523 (pt 8)		1				
5-13-11-9-490 (pt 197)	2	1				
Unknown	2 (135 & 135a)			1 (135)		1 (135)

Appendix 7. *Salmonella* prevalence on egg farms by production system

Of the 49 egg farms included in the study, just over 60% (30/49) used free-range system. About 30% (15/49) used a cage-based production system and the remaining 8% of businesses (4/49) used a barn system. Overall, businesses in this survey produced approximately 1.3 million eggs per day. Further analysis of the data found that free-range and cage eggs were produced in roughly equal quantities—640,000 and 600,000 eggs/day, respectively. Fewer barn-laid eggs (86,000 eggs/day) were produced by businesses included in the survey.

Types of production systems observed in the survey

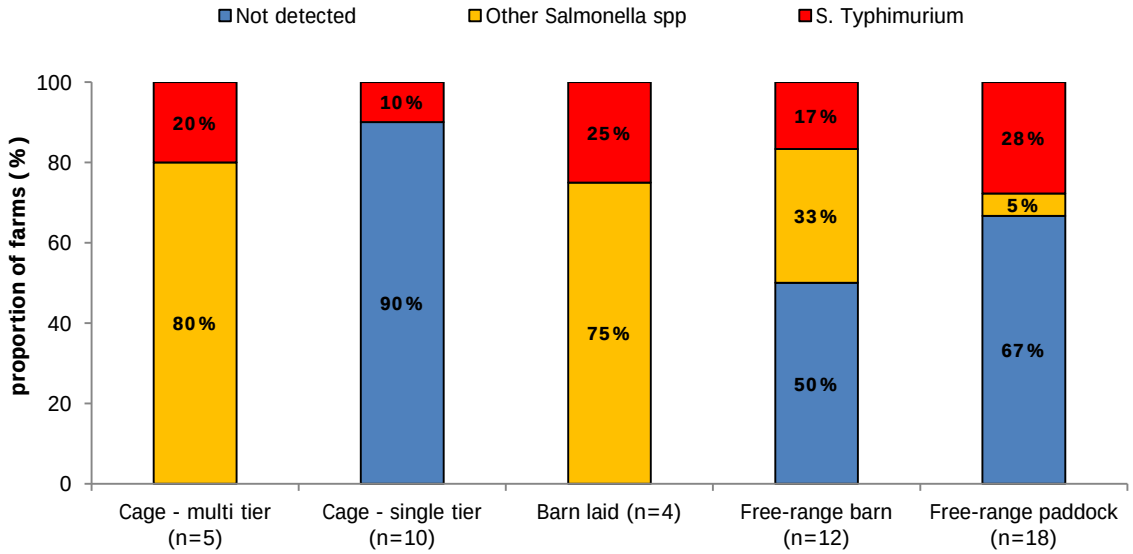
Free range systems	Cage systems
Free-range barn – where the hens are housed in one large shed. They have access to food and water and they can lay their eggs in a long central box in the shed while having access to an outdoor area for sometime each day. Usually a central conveyer belt under the laying box removes the eggs from the shed. There can be between 6,500 and 100,000 hens in each shed depending on the shed dimensions.	**Cage single tier** – where cages are only on one level and faecal matter accumulates on the floor under the cages. The number of hens often varies between 250 and 10,000 per shed.
Free-range paddock – the hens are housed in small movable sheds. They lay their eggs in single boxes in the sheds and usually the eggs are collected by hand. Food and water is usually supplied in semi-enclosed troughs in the paddocks. The number of sheds per paddock can vary, however there can be between 240 and 1,500 hens for each movable shed.	**Cage multi-tier** – where cages are tiered up to 8 tiers high and faecal matter, feed and egg collection is usually automated via a conveyer belt system. There can be between 10,000 and 92,000 hens per shed depending on the shed dimensions and the number of tiers.

Salmonella was found across all production systems

The figure below presents differences in *Salmonella* prevalence across production system categories in the farms surveyed. Multi-tier cage system and barn-based systems had the highest *Salmonella* prevalence. However, making causal inferences about the effect of production system on *Salmonella* prevalence in egg farms is not justified by the survey design.

Aggregating the data across production systems, *Salmonella* prevalence for both cage-based and free-range systems were 40% (6/15 and 12/30 respectively). It is shown that single-tier cage farms included in this survey had the lowest *Salmonella* prevalence (10%, 1/10), compared to any other category. This was followed by farms with free-range, paddock-based systems (34%, 6/18). In contrast, all multi-tier cage (5/5) and barn-based (4/4) production systems in the study were positive for *Salmonella*. Finally, the results showed that half (6/12) of the barn-based, free-range farms were positive for *Salmonella*.

Proportion of *Salmonella* positive farms in this study according to production system



Published scientific literature was examined for further information on the possible effects of production system on *Salmonella* prevalence. Holt et al. (2011) stated that it is unclear whether or how different production systems affect on-farm *Salmonella* infection rates, but that testing of layer flocks in the EU showed a higher prevalence of *Salmonella* in flocks housed in conventional cages compared to those housed on the floor.

As explanation, Holt et al. (2011) highlighted the potential difficulties for farmers to effectively access and clean the cages/sheds and drinkers in cage-based systems, noting that, due to the restricted hen movement, cage houses are potentially a more attractive location for *Salmonella*-carrying rodents. Even so, Holt et al. (2011) warned that the higher incidence of *Salmonella* in cage facilities may be a reflection of sampling logistics and faeces, and their resident salmonellae are localised in manure pits beneath the cages rather than being disseminated over a wide area in barn-style or free-range facilities.

Conversely, other researchers have detected a lower incidence of *Salmonella* in conventional cage systems than cage-free systems (cited in Holt et al., 2011). Free-range housing, in which the hens spend a portion of their time outdoors, increases their interactions with wildlife, which can increase the likelihood of *Salmonella* contamination. In addition, the soil environment contaminated by free-range flocks is difficult to disinfect and could serve as a persistent source of *Salmonella* for future birds raised in that facility.

In the survey, Authority officers encountered many farms with deep manure pits which were difficult to access, and did not facilitate the cleaning and disinfection of the manure pits. It is possible that due to difficulties with cleaning in between flocks and the additional complexity of the cage systems (stacked cages, drinkers, and manure belts), there is greater potential for carryover of *Salmonella* from flock to flock (Carrique-Mas et al., 2009; Higgins et al., 1982).

Appendix 8. *Salmonella* prevalence on egg farms by production volume

For the purposes of the survey, business size classifications were based on daily egg production volumes. The table below includes an analysis of the proportion of businesses sampled based on size and production system. The majority of egg farms in the survey produced between 1000 and 30,000 eggs/day (most likely to be in the 'small' licence category) and most were likely to produce eggs under a free-range production system. As identified in the corresponding industry profile (NSW Food Authority, 2012), free-range was found to be the most common egg production system compared to cage and barn-based systems. It is worthwhile noting that businesses producing less than 35 eggs each day (<20 dozen eggs/week) were excluded from the survey as they do not require licences under the Regulation.

Proportion of businesses sampled by production system and production size

Production system	Number of eggs produced/day				
	<1000*	1,000-10,000	10,000-30,000	30,000-100,000	100,000-250,000
Free-range	17% (5/30)	40% (12/30)	30% (9/30)	7% (2/30)	7% (2/30)
Barn-based		25% (1/4)	75% (3/4)		
Cage	7% (1/15)	60% (9/15)	7% (1/15)	13% (2/15)	13% (2/15)

Salmonella was found across farms with different production volume

As seen from the figure below, higher prevalence of *Salmonella* was found on farms producing greater volumes of eggs compared to farms producing fewer eggs. *S.* Typhimurium was also detected in egg farms regardless of their production volumes. As it is impossible to exclude the effect of season, production system, feed source and region as a cause of difference, we cannot infer that this observed difference between the categories is the result of size. In other words, it is not possible, from this dataset, to infer that farm size (egg production volume) causes differences in *Salmonella* prevalence. However, the findings do provide an important foundation for informing the design of surveys in the future.

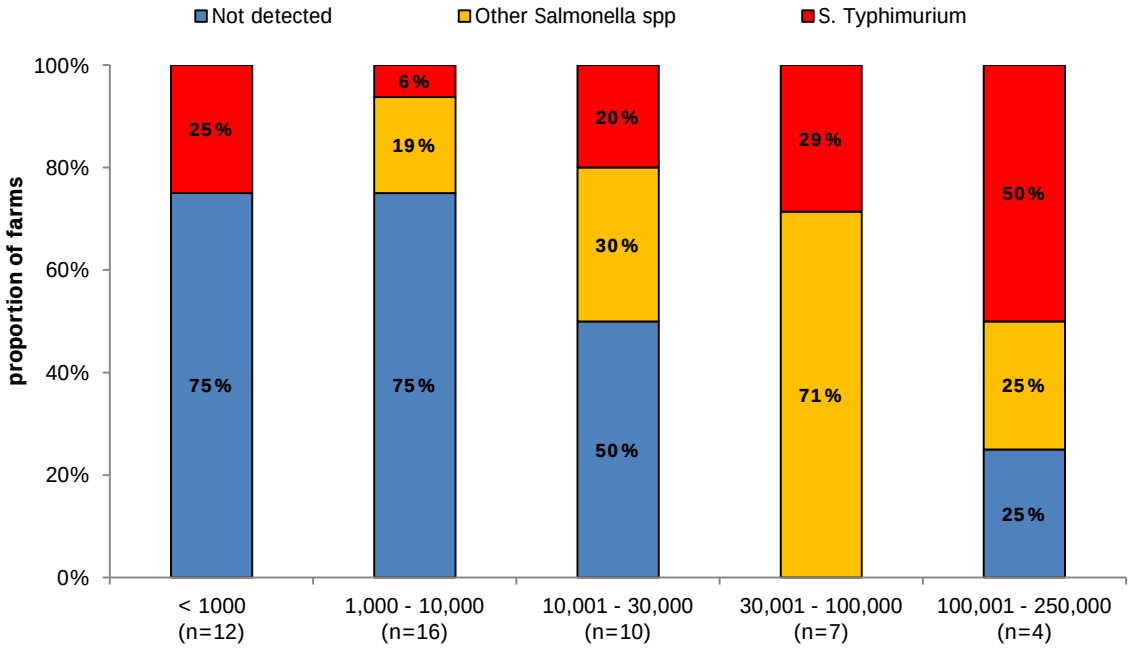
The survey found that small-volume farms (producing less than 1000 eggs/day) had *Salmonella* prevalence of 25% (3/12 positive) and all samples tested positive for *S.* Typhimurium. Data from the baseline profiling survey indicated that about 25% (32/121) of businesses included in the study were producing less than 1000 eggs per day (NSW Food Authority, 2012). Therefore, about 38% (12/32) of farms of this size were included in the microbiological survey.

Of the businesses producing slightly larger egg volumes (1000 to 10,000 eggs per day), similar *Salmonella* prevalence (25%, 4/16) was detected. At the time, the baseline profiling survey identified that 36% (44/121) of businesses produced egg volumes in this range (NSW Food Authority, 2012). Therefore, just over one-third (16/44) of farms of this size were sampled.

The survey found that 50% (5/10) of businesses producing volumes of eggs between 10,000 and 30,000 eggs/day were positive for *Salmonella.* The baseline profiling survey found that about 26 businesses (26/121) were producing volumes of this range. Therefore, almost 40% of farms of this size were included in the microbiological survey (NSW Food Authority, 2012).

Finally, the figure below illustrates that 91% (10/11) of businesses producing the largest volumes of eggs (over 30,000 eggs per day) were positive for *Salmonella* (the last two columns). Data from the profiling survey indicated that approximately 16% of businesses (20/121) produced greater than 30,000 eggs per day (NSW Food Authority, 2012). It is worth noting that over half (11/20) were sampled as part of this microbiological survey.

Proportion of *Salmonella* positive farms in this study according to daily egg production volumes



A review of the published scientific literature offers insight into the likelihood of *Salmonella* prevalence in relation to farm size (egg production volumes). A study by Angen et al. (1996) found there was a significantly increased risk of *Salmonella* contamination of flocks if there were more than three houses/sheds on a farm. The researchers theorised that an increased number of houses/sheds on the farm might increase the possibility of transmission between houses. They also claimed that more sheds meant less time for cleaning and disinfection of the sheds before the introduction of new stock.

A large scale USA study revealed that flock size is another factor potentially affecting the prevalence of *Salmonella* in the egg laying environment. In that study, houses containing more than 100,000 layers were four times more likely to be environmentally positive for *Salmonella* Enteritidis than similar houses containing fewer than 100,000 hens (USDA/APHIS, 2000). Possible explanations for this increase included the fact that prevalence may be due to the higher densities of birds in these facilities producing increased volumes of contaminated faeces and dust.

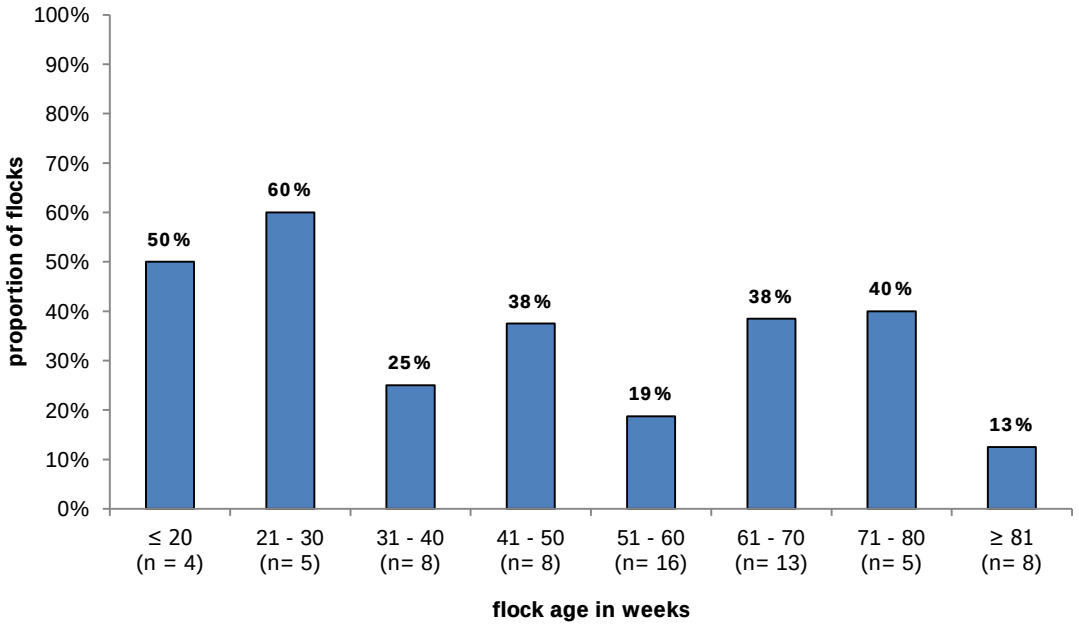
Appendix 9. *Salmonella* prevalence on egg farms by flock age

As flocks age, some scientific studies suggest that hens become less susceptible to infection by environmentally acquired *Salmonella* (Linton et al., 1985; Wales et al., 2007) and/or are less likely to shed *Salmonella* (Renwick et al., 1992).

The figure below presents the proportion of *Salmonella* positive flocks for eight flock age categories to check for any obvious signs of confounding due to flock age. The data was obtained from 67 sheds included in this survey housing a single age flock. A flock was categorised positive if at least one environmental sample (boot/cage swabs or faecal material) was positive for *Salmonella*.

Salmonella was detected across flocks of all ages but prevalence was highest for the youngest flock age categories—< 20 weeks and 21–30 weeks. No obvious signs of confounding due to flock age were identified. Overall, no apparent relationship can be identified between prevalence and flock age but it is important to note that sampling was not designed to assess the effect of flock age. From this data set, flock age cannot be inferred as a cause of difference in the *Salmonella* prevalence rates. However, for future studies, it is important to reference the flock age profile of the sample population.

Proportion of *Salmonella* positive flock per age category for egg farms surveyed in NSW



Appendix 10. *Salmonella* prevalence on egg farms by season

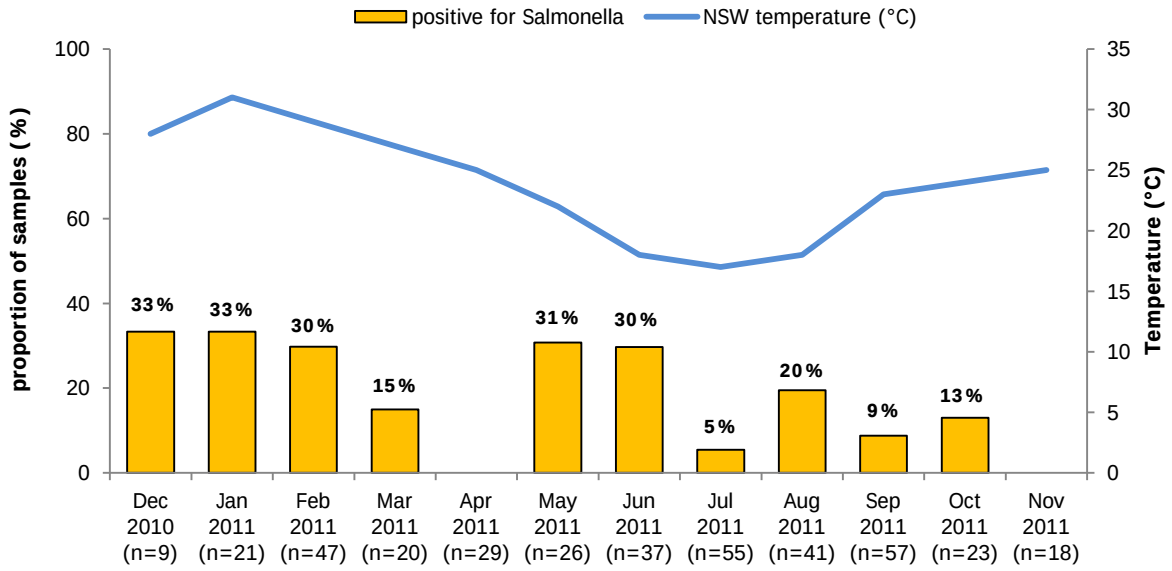
The figure below plots the proportion of positive *Salmonella* samples against the average temperature in NSW for each month during the sampling period. Overall, as temperatures declined, no obvious decrease in *Salmonella* prevalence was observed. However, upon analysing the data on a seasonal basis, higher Salmonella prevalence was observed for summer compared with any other season. It is important to highlight that sampling was not designed to assess the effect of weather on *Salmonella* prevalence and therefore the effect of season cannot be inferred as a cause of difference.

There are opportunities for future work examining the effect of seasonality as there are varying views in the scientific literature. In some published reports, it is expected that higher environmental temperatures in summer increase bird stress and bacterial multiplication rates, resulting in higher levels of hen house contamination (Wales et al., 2007). Another study noted that the fly population increases during summer and this increases the likelihood of transmitting *Salmonella* within the laying shed (Olsen & Hammack, 2000).

Alternatively, Angen et al. (1996) found that autumn had the highest risk of *Salmonella* infection in hens due to the difficulty with cleaning, disinfection and drying during cold season.

Finally, two studies conducted in the UK and Belgium found no seasonal effect on egg contamination rates (Davies & Breslin, 2004; Namata et al., 2008). The studies contend that well-designed and well-insulated hen houses should not be subject to excessive temperature fluctuations at any time of the year, so a seasonal effect may be more marked in accommodation that has serious deficiencies in ventilation and insulation.

Salmonella prevalence in samples taken each month and average monthly temperatures in 2011

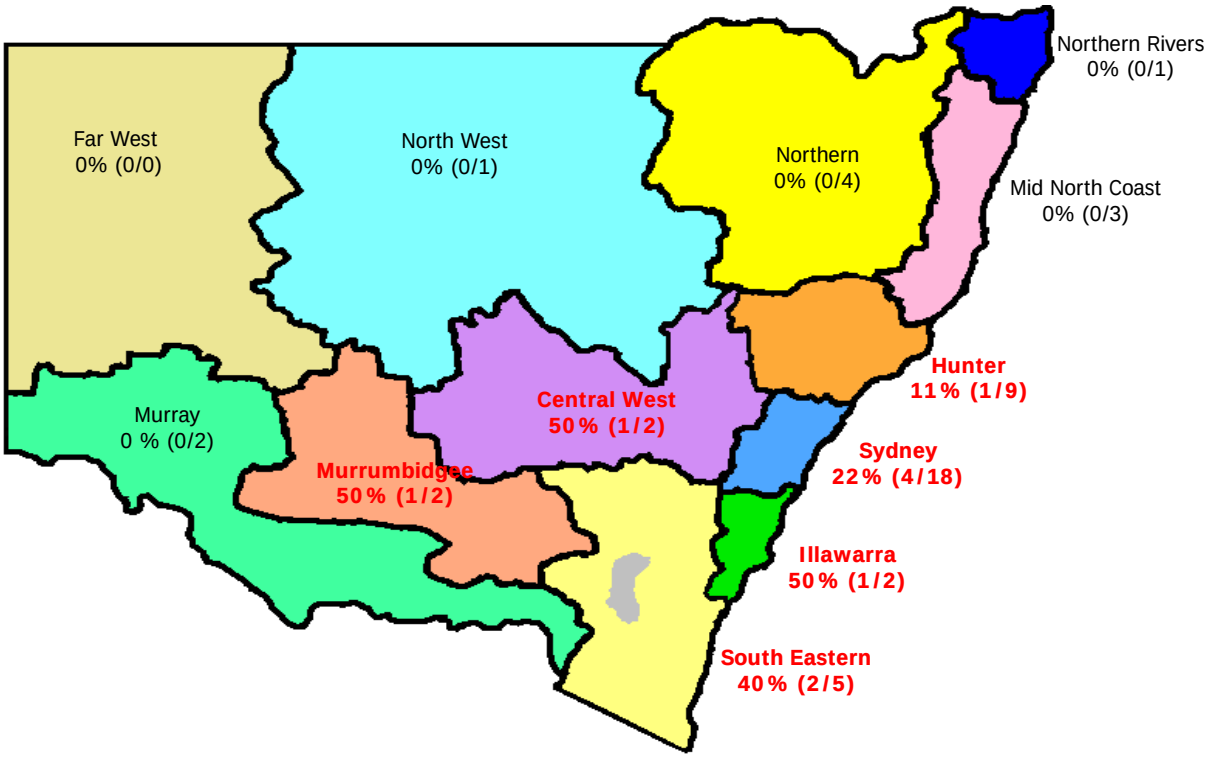


Seasons	*Salmonella* positive samples
Summer (Dec–Feb)	31% (24/77)
Autumn (Mar–May)	15% (11/75)
Winter (Jun–Aug)	17% (22/133)
Spring (Sep–Nov)	8% (8/98)

Appendix 11. *S.* Typhimurium detection and egg farm location

The figure below is a map of NSW illustrating *S.* Typhimurium detection on a regional basis. It shows that samples were taken from farms located in all but one region across NSW.

As shown below, *S.* Typhimurium was detected in four out of the six coastal regions in NSW. It is worth noting that just over 60% of all farms licensed at the time were located within these four regions. However, due to the fact that the number of farms within some regions was small, it is impossible to make any inferences in relation to prevalence of *S.* Typhimurium and location.



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