

Baseline survey on the prevalence and concentration of *Salmonella* and *Campylobacter* in chicken meat on-farm and at primary processing

A survey conducted under the Implementation Subcommittee Coordinated Food Survey Plan with participation from food regulatory jurisdictions in New South Wales, Queensland, Western Australia, South Australia and Tasmania.

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 - Safe Food Production Queensland
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1. Executive Summary

Food Standards Australia New Zealand was the lead agency for a national coordinated survey to collect baseline data on the prevalence and concentration of *Salmonella* and *Campylobacter* on poultry and poultry meat at various stages along the supply chain. As part of its through-chain approach to food regulation, FSANZ has developed a Primary Production and Processing Standard for Poultry Meat (Standard for Poultry Meat). The overall objective of this Standard is to reduce the likelihood of illness occurring from the consumption of poultry, with a focus on measures to reduce both the prevalence and concentration of *Salmonella* and *Campylobacter* on poultry meat. These two pathogens are considered to be the main microbiological hazards associated with the consumption of poultry meat. Comparison of results from this baseline survey with those from a follow up survey, to be undertaken after the Standard for Poultry Meat is fully implemented, will be used to assess the effectiveness of the new Standard.

The states that participated in at least one component of this survey were New South Wales, Queensland, Western Australia, South Australia and Tasmania. Western Australia was the only jurisdiction to participate in all three components of the survey. Neither of the territories participated, as they do not have poultry meat farms or processors within their jurisdictions.

This study measured both the prevalence and where appropriate, concentration, of *Salmonella* and *Campylobacter* at three points along the poultry meat supply chain, on-farm, just prior to processing and at the end of primary processing. Microbiological samples were collected during 2007 and 2008. All samples were collected from the chicken meat industry and the on-farm surveys were conducted on chicken meat farms. While the Standard for Poultry Meat will apply to poultry other than chicken, chicken meat is consumed in far greater quantities than other poultry.

Samples that were positive for *Salmonella* were typed to determine the serovars. The most common serovar was Sofia, except in Western Australia where Typhimurium was the most common. Sofia is considered to have low virulence to humans (i.e. rarely implicated in cases of human salmonellosis). Nationally, the most common non-Sofia serovar was Typhimurium, followed by Infantis and Mbandaka.

The results from this survey were compared with recent overseas data on the prevalence and/or levels of *Salmonella* and *Campylobacter* in poultry. However, caution needs to be taken when comparing these data due to the differences in survey design, methodology and sample sizes and therefore the comparisons are somewhat rudimentary. Comparisons were made with similar baseline surveys, and also with studies where specific interventions are in place to lower flock prevalence of *Campylobacter* and/or *Salmonella*.

On farm

As an indicator of flock prevalence of *Salmonella* and *Campylobacter*, pooled faecal samples were collected from farms in Western Australia before first pick-up¹. A total of 233 pooled faecal samples were collected from 39 farms. Of the 233 samples collected, 46.8% were positive for *Salmonella* (comprised of 46.8% positive for non-Sofia serovars and 0.9% positive for Sofia, with some samples having multiple serovars²) and 64.4% were positive for *Campylobacter*. In 21 sheds (9%), *Salmonella* and *Campylobacter* were not detected.

In some farms *Salmonella* or *Campylobacter* were not detected (15.4% and 28%, respectively) but there were no farms where both pathogens were not detected, with at least one shed testing positive for *Salmonella* or *Campylobacter* on every farm.

Currently in Australia there are no regulatory measures in place to lower the prevalence of these two pathogens in poultry flocks, however, the majority of poultry growers comply with an industry biosecurity manual, to minimise the introduction and spread of infectious diseases generally in poultry flocks.

The results were compared with two similar on-farm baseline surveys undertaken in Canada and Ireland. The prevalence of *Campylobacter* positive flocks was higher in this survey (64.4%) compared to 35% in Canada. For *Salmonella*, the prevalence was very similar to Canada (46.8% compared to 50% in Canada) but higher than Ireland where the prevalence was 27%. A study in the Netherlands reported lower prevalences of *Salmonella* and *Campylobacter* flock prevalence. However, specific interventions were in place to control these two pathogens.

An on-farm biosecurity/food safety survey was also conducted to investigate the uptake and implementation of on-farm biosecurity measures to control *Salmonella* and *Campylobacter* infection of flocks. Forty-eight on-farm surveys were completed, 38 in Western Australia and 10 in Tasmania. The farms surveyed account for approximately 90% of broiler meat produced in Western Australia and Tasmania. The results of this survey will be presented in a separate report.

Prior to processing

To determine the prevalence of *Salmonella* and *Campylobacter* in poultry entering processing facilities, caecal³ contents of poultry were collected for testing. The concentration of *Salmonella* and *Campylobacter* in the caeca was also quantified for positive samples. This quantification can give an indication of the frequency of 'high-shedders' (i.e. percentage of birds with high concentrations of the pathogens in their caeca).

¹ Chicks are placed in the broiler grow-out farm at the age of approximately 1 day and remain on the farm until they reach slaughter weight, which is usually between 30 and 60 days. At this point depopulation occurs whereby all, or a proportion, of birds are removed and transported to the slaughter facility for processing.

² The two samples that were positive for Sofia were also positive for Give.

³ In poultry, the caeca are two blind-ended tubes at the junction of the small and large intestines, where undigested food particles are subjected to microbial breakdown.

South Australia and Western Australia participated in this component of the survey, with a total of 636 caecal samples collected. Overall, 12.7% of samples tested positive for *Salmonella*, with 7.5% positive for non-Sofia serovars and 5.2% for Sofia. Multiple *Salmonella* serovars were isolated from some samples. The mean concentration of *Salmonella* was low, 1.02 log₁₀ MPN/gm. For *Campylobacter*, the average prevalence was 84% and the mean concentration was 6.87 log₁₀ cfu/gm.

A survey in Iceland tested the incoming flock prevalence of broilers for *Campylobacter* by testing the contents of the caeca just prior to slaughter. The flock prevalence was low (15%), which is comparable to that of other northern European countries. Additionally, Iceland has an official policy of testing flocks prior to slaughter and requiring the meat derived from *Campylobacter* positive flocks to be sold frozen, which motivates the industry to lower the flock prevalence.

Post primary processing

To determine the prevalence and concentration of *Salmonella* and *Campylobacter*, post processing, rinse samples from whole carcasses were taken from processing plants, following the chilling step.

A total of 1112 carcass rinse samples were collected from four states (NSW, Qld, SA and WA). For *Salmonella*, 36.7% of carcass rinse samples were positive with 22.1% positive for non-Sofia serovars and 15.1% for Sofia. Multiple *Salmonella* serovars were isolated from some samples. The *Salmonella* concentration on positive samples was on average -1.99 log₁₀ MPN/cm² or about 1 per 100cm². For *Campylobacter*, 84.3% of carcass rinse samples were positive. In Western Australia and New South Wales, the *Campylobacter* concentration of positive samples was, on average, 0.70 log₁₀ cfu/cm² or ~500 cfu/100cm². In Queensland, where only counts >100 cfu/ml were quantified, the mean concentration was 1.45 log₁₀ cfu/cm² or ~2818 cfu/100cm².

The results of this survey were compared with similar baseline surveys. In a study conducted in Canada, the prevalence of both *Salmonella* (37.5%) and *Campylobacter* (75%) were similar to the results from this survey (*Salmonella* 36.7% and *Campylobacter*, 84.3%). *Campylobacter* results were also similar to a baseline study conducted in the US in 1994-1995, where *Campylobacter* prevalence was reported at 88.2%. The prevalence of *Campylobacter* from this survey was higher than a baseline level of 70%, estimated for the UK in 1995, based on the available surveillance data. The *Salmonella* results from this survey were higher than those found in another baseline study conducted in the United States (20%).

The *Salmonella* and *Campylobacter* results from this survey are higher than those found in studies conducted after specific interventions strategies have been implemented. If the results are compared with New Zealand for *Campylobacter*, Australia has a much higher prevalence (NZ prevalence was 30.6% in second quarter of 2008) and higher concentrations. New Zealand has successfully lowered both the prevalence and concentrations of *Campylobacter* in poultry since implementing a *Campylobacter* reduction strategy in 2006 and setting poultry processing targets in 2008.

Of the 1112 rinse samples taken, 790 were tested for Total Viable Counts (TVC) and *Escherichia coli*. Unsatisfactory results can indicate that a step in the poultry processing is not performing as expected and corrective action may need to be taken. For TVC, almost 100% of the samples were considered excellent or good and none were marginal or poor, according to the performance criteria. For *E. coli*, 94.5% of samples were classified as excellent or good, and the remainder were all acceptable. In light of the results for *Salmonella* and *Campylobacter*, the testing of these indicator organisms in poultry at the end of the processing is not a good indicator of the likelihood of the poultry being contaminated with these two pathogens.

This survey has provided baseline data on the prevalence and levels of *Salmonella* and *Campylobacter* on chicken meat at both the primary production and primary processing stages of the chicken meat supply chain. The results from farms in Western Australia, indicate that poultry are being infected with *Salmonella* and *Campylobacter* on farm. The results from Western Australia and South Australia indicate that a large percentage of the live poultry entering the processing plants are infected with *Campylobacter* (84%) and to a much lesser extent, *Salmonella* (12.7%). At the end of primary processing, the prevalence of poultry contaminated with *Campylobacter* remained relatively constant (84%). However, there was an increase in the prevalence of *Salmonella* (36.7% total *Salmonella*, of which 22.1% were positive for non-Sofia serovars).

The results from the samples taken at the end of primary processing are similar to the results from the retail baseline microbiological survey carried out in 2005/2006 in South Australia and New South Wales. The results from this survey showed that 43.3% of chicken samples tested were positive for *Salmonella* (12.8% being non-Sofia) and approximately 90% were positive for *Campylobacter coli/jejuni* (Pointon et al, 2008). The mean counts were also similar to the mean counts found in this survey⁴. This indicates that the prevalence and levels of *Salmonella* and *Campylobacter* on chicken carcasses post processing, is similar to that found on the fresh chicken purchased by consumers.

When compared with results from similar baseline surveys overseas, the results from this survey were generally similar to those conducted in the US, UK and Canada. With the exception of the UK, countries that have put in place specific intervention strategies to lower *Salmonella* and *Campylobacter* in poultry, have achieved significant reductions.

2. Introduction

As part of its through-chain approach to food regulation, FSANZ is developing a Primary Production and Processing Standard for Poultry Meat (Standard for Poultry Meat). This Standard is being developed in consultation with a Standard Development Committee, comprising representatives from the poultry industry, government agencies and a consumer representative.

⁴ In the retail survey the mean counts were -1.42 to -1.6 log MPN/cm² for *Salmonella* and 0.78 to 0.87 log cfu/cm² for *Campylobacter*.

A scientific risk assessment of the public health and safety of poultry meat in Australia was carried out as the first step in the development of the Standard for Poultry Meat. This scientific assessment concluded that the main microbiological hazards associated with poultry meat are contamination with *Salmonella* and *Campylobacter* and that the prevalence and concentration of contamination by *Salmonella* and *Campylobacter* species in poultry were affected by a range of factors at the primary production, processing, retail and consumer stages of the poultry meat supply chain. The scientific assessment also concluded that there was reasonable evidence to indicate poultry was a vehicle for a proportion of salmonellosis and *Campylobacteriosis* cases in Australia (FSANZ, 2005).

Salmonellosis is a notifiable disease in Australia. *Campylobacteriosis* is also a notifiable disease in all states and territories except New South Wales, where it is only notifiable in the case of an outbreak. *Campylobacteriosis* is the most commonly notified food-borne illness in Australia and it is estimated that approximately 30% of cases (or 83,100 cases per year⁵) could be attributed to contaminated poultry meat (Stafford et al, 2007). Salmonellosis is the second most frequently notified illness at 9,484 notifications or 45 cases per 100,000 population (The OzFoodNet Working Group, 2008).

Based on the outcomes of the scientific assessment, risk management strategies have been proposed to address the identified hazards. In general terms, the draft Standard for Poultry Meat proposes that poultry primary producers and processors identify and manage the hazards in their businesses. The overall objective is that poultry businesses put in place strategies to reduce the likelihood of illness occurring from the consumption of poultry and in particular, reduce the prevalence and concentration of *Salmonella* and *Campylobacter* in poultry meat.

The Standard for Poultry Meat is expected to be finalised in 2010 and businesses will then have two years to comply with the new Standard. It will apply to all businesses involved in the growing or processing of poultry intended for sale for human consumption.

FSANZ is committed to undertaking a scientific and technical evaluation of the impact of implementing new food regulatory measures. The aim of the evaluation is to assess the effectiveness and appropriateness of food regulatory measures by assessing the long term impact on stakeholders and to provide evidence to inform future decisions on food regulation. As the Standard for Poultry Meat is a proposed new regulatory measure, FSANZ will be evaluating its impact. To evaluate the impact of a new regulatory measure, benchmarking data must be collected before the measure is introduced so that a comparison can be made when the regulatory measure is in place. As part of the FSANZ 2004-2008 Evaluation Strategy, FSANZ commissioned benchmark research on the poultry meat industry. This research collected data on awareness, knowledge and behaviour of poultry meat businesses, government officers and consumers in relation to food safety issues (Colmar Brunton Social Research, 2005).

⁵ This is 30% of the estimated 277,000 total cases of campylobacteriosis that occurs each year in Australia (Hall et al, 2005).

Complementary to this research, a retail baseline microbiological survey was carried out in 2005-06 jointly by South Australia (PIRSA/SARDI⁶), New South Wales (NSWFA), the Australian Government Department of Health and Ageing and FSANZ to estimate the prevalence and concentration of *Salmonella* and *Campylobacter* contamination of chicken products available at retail in New South Wales and South Australia. The results from this survey showed that 43.3% of chicken samples tested were positive for *Salmonella* (12.8% being non-Sofia) and approximately 90% were positive for *Campylobacter coli/jejuni* (Pointon et al, 2008).

During the development of the Standard for Poultry Meat, concerns have been raised about the lack of data on the current microbiological status of poultry meat along the supply chain, particularly chicken, as chicken is by far the main type of poultry meat consumed in Australia. While the retail survey provides data on the microbiological status of chicken at the end of the supply chain, only limited data is available at other parts of the chain. If data can be obtained on the microbiological status of chicken along the entire supply chain, this will assist in:

- assessing the effectiveness of the proposed regulatory measures in the Standard for Poultry Meat for the different parts of this chain
- identifying stages along the supply chain that most impact on *Salmonella* and *Campylobacter* prevalence and concentration.

A proposal to obtain baseline data on the prevalence and concentration of *Salmonella* and *Campylobacter* along the poultry meat supply chain was considered at the Implementation Subcommittee (ISC) Coordinated Food Survey Plan Workshop on 6 April 2006. At this meeting, it was agreed that the proposal should be progressed through the ISC Coordinated Food Survey Plan and a project team formed to assist with the proposed survey.

The project team recommended that the prevalence and (where appropriate) concentration of *Campylobacter* and *Salmonella* be tested on-farm, prior to processing and at the end of processing. Four states participated in this part of the survey (WA, NSW, Qld and SA). An on-farm survey was also recommended to investigate the uptake and implementation of on-farm biosecurity measures to control *Salmonella* and *Campylobacter* infection of flocks. This was carried out by WA and Tas. The two territories (ACT and NT) did not participate as their jurisdictions do not have poultry meat farms or processors.

This report presents and discusses the results from the microbiological testing of *Campylobacter* and *Salmonella* on-farm, prior to processing and at the end of processing. The results from the on-farm survey will be presented in a separate report.

⁶ Department of Primary Industries and Resources South Australia and the South Australian Research and Development Institute

3. Survey objective

This survey aimed to collect baseline data on the prevalence and concentration of *Salmonella* and *Campylobacter* species on poultry and poultry meat at various stages along the supply chain prior to the implementation of the Standard for Poultry Meat. Comparison of results from this baseline survey with those from a follow-up survey, to be undertaken after the Standard for Poultry Meat has been fully implemented, will be used to assess the effectiveness of the Standard for Poultry Meat.

The main objectives of the baseline survey were:

- 1) to establish the flock prevalence of *Salmonella* and *Campylobacter* on poultry by collecting samples on-farm
- 2) to investigate the uptake and implementation of on-farm biosecurity measures to control *Salmonella* and *Campylobacter* infection of flocks
- 3) to establish the prevalence and concentration of *Salmonella* and *Campylobacter* infected poultry entering primary processing
- 4) to establish the prevalence and concentration of *Salmonella* and *Campylobacter* on poultry carcasses by collecting samples at the end of primary processing.

4. ISC Coordinated Food Survey

On 30 October 2003, the Food Regulation Standing Committee's Implementation Subcommittee (ISC) agreed to the development of a 'Coordinated Food Survey Plan' for the Australian jurisdictions, food regulatory partners and New Zealand. This was in recognition that there were significant advantages in implementing agreed national survey priorities in a prospective and coordinated manner. A national coordinated survey to obtain baseline data on the prevalence and concentration of *Salmonella* and *Campylobacter* along the poultry meat supply chain was agreed to by ISC in April 2006.

To progress the survey, a project team was formed with representatives from the participating jurisdictions, the Australian Government Department of Health and Ageing, the South Australian Research and Development Institute (SARDI) and FSANZ. FSANZ was nominated as the lead agency and was responsible for coordinating the baseline survey. The participating jurisdictions were:

- New South Wales Food Authority
- Department of Health Western Australia
- Safe Food Production Queensland
- Department of Primary Industries and Water Tasmania
- Department of Primary Industries and Resources South Australia.

As well as participating as a member of the survey team, SARDI was contracted to undertake statistical analysis and assist with report preparation.

The Poultry Survey Project Team was responsible for developing a survey proposal and for implementing this proposal. The participating jurisdictions, in accordance with the survey proposal, collected and tested samples and conducted on-farm surveys. The Project Team then assisted with the compilation and analysis of the results and the writing of this report.

The Poultry Survey Project Team communicated throughout the survey period with the poultry industry, via the FSANZ Standard Development Committee on Poultry Meat. This survey received excellent cooperation and assistance from the poultry industry. The Poultry Survey Project Team is grateful to the poultry industry for providing access for relevant government officers to collect samples from poultry farms and processing plants. The Project Team would also like to specifically thank the poultry farms that participated in the on-farm biosecurity/food safety survey.

5. Resources spent on the survey

This survey was financed from contributions from the participating jurisdictions (~\$224,000), DoHA (\$110,000) and FSANZ (\$66,000). The money from DoHA and FSANZ was distributed to the participating jurisdictions to assist with the financing of the survey. Apart from providing a significant financial contribution, the jurisdictions also contributed many hours of staff time. In particular, the Department of Health, Western Australia's contribution was large as it was the only state to participate in all three components of the survey. Microbiological analysis of the samples was conducted by the state laboratories. The poultry industry also provided personnel time to assist with the survey. The total cost of the survey, when considering cash inputs, government and industry personnel time and other expenses such as teleconference and travel costs was approximately \$500 000. This information may assist future planning of similar national surveys for *Campylobacter* and *Salmonella*.

6. Survey design and methodology

The survey was designed to measure both the prevalence and where appropriate, concentration, of *Salmonella* and *Campylobacter* at three points along the poultry supply chain - on farm, just prior to processing and at the end of primary processing. An on-farm biosecurity/food safety survey was also developed to investigate the uptake and implementation of on-farm biosecurity measures to control *Salmonella* and *Campylobacter* infection of flocks.

All microbiological samples were tested initially for absence/presence of *Campylobacter* and *Salmonella*, within the limit of detection. Positive caecal and carcass rinse samples were enumerated. Positive *Salmonella* samples were typed to determine the serovars. Results are reported for 'total *Salmonella*' and '*Salmonella* non-Sofia serovars'. All isolated *Salmonella* serovars are also listed in a table at Attachment 4. *Campylobacter* positives were not typed and therefore all results are for total *Campylobacter* spp.

All samples for this survey were collected from the chicken meat industry. While the Standard for Poultry Meat defines poultry as chicken, turkey, duck, squab (pigeons), geese, pheasants, quail, guinea fowl and other avian species (except ratites), chicken meat is

consumed in far greater quantities than other poultry. Per capita consumption of chicken meat was estimated to have reached 37 kg/person in 2007/2008, compared to other poultry being 2.2 kg/person (ACMF, 2009).

Officers from the jurisdictions that participated in this survey were requested to comply with the biosecurity measures on the farms being visited.

Each participating jurisdiction managed the way in which samples were collected over time to suit the constraints of the participating laboratory. In South Australia samples were collected from March – May 2007, in Western Australia, samples were collected from October 2007 until March 2008, in New South Wales from March – July 2008 and in Queensland from April – August 2008.

6.1 On-farm

6.1.1 Faecal samples

Western Australia was the only state to participate in this component of the survey. Fresh faecal droppings were collected from farms just before the first pick-up.⁷ The droppings were collected before the first pick-up so that the results could be compared across the sheds.

To determine the numbers of farms that needed to be tested, a prevalence estimate of 50%⁸ for both *Salmonella* and *Campylobacter* was used. The minimum number of farms needed to be sampled to detect a prevalence of this level was then determined. Two hundred and thirty three samples were collected from thirty-nine farms.

Samples were pooled to obtain at least 250gm. Information was recorded at the time of collection in accordance with Form A: Sample Collection On-Farm (Attachment 1). Samples were analysed to determine prevalence of *Campylobacter* and *Salmonella* on farm.

Upon receipt of the samples at the laboratory, samples were thoroughly mixed and analysed in accordance with the following methods.

Campylobacter

Faecal droppings were cultured directly onto agar media plates as described in AS 5013.6 – 2004 “Food Microbiology Method 6: Examination for specific organisms – *Campylobacter*”

Salmonella

Twenty-five grams of the thoroughly mixed faecal droppings were cultured as per “AS 5013.10 - 2004: Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp”.

For further detail on the methods used for the confirmation of *Salmonella* and *Campylobacter*, see Attachment 2: Method of analysis for *Salmonella* and *Campylobacter*.

⁷ Chicks are placed in the broiler grow-out farm at the age of approximately 1 day and remain on the farm until they reach slaughter weight, which is usually between 30 and 60 days. At this point depopulation occurs whereby all, or a proportion, of birds are removed and transported to the slaughter facility for processing.

⁸ This is a worst case scenario used to calculate the number of samples required to statistically compare results.

6.1.2 Survey on biosecurity and food safety practices

A survey was also conducted to investigate the uptake and implementation of on-farm biosecurity measures to control *Salmonella* and *Campylobacter* infection of flocks. The participating farms were asked to complete a specifically designed questionnaire.

Western Australia and Tasmania participated in this component of the survey. Forty-eight on-farm surveys were completed; 38 in Western Australia and 10 in Tasmania. In Tasmania, the 10 farms surveyed produce approximately 90% of broiler meat produced in Tasmania. Similarly in Western Australia, the 38 farms surveyed represent approximately 90% of the broiler meat industry in Western Australia. As this was a qualitative survey with no microbiological component, the results of the survey will be presented in a separate report.

6.2 Prior to processing

To determine the prevalence of *Salmonella* and *Campylobacter* in poultry entering processing facilities, the caecal contents of poultry were randomly collected for testing. In poultry, the caeca are two blind-ended tubes at the junction of the small and large intestines, where undigested food particles are subjected to microbial breakdown. In chicken, the primary site of colonisation of *Campylobacter* is the lower gastrointestinal tract, especially the caeca (Beery et al, 1988).

South Australia and Western Australia participated in this component of the survey. A proportional sampling approach was utilised within each state based on throughput at each plant and sample numbers based on the expected prevalence of *Salmonella* and *Campylobacter* in that state. Information was obtained about each state's poultry industry, including the number of major processors, the estimated throughput at each of these processors and an estimate of the *Salmonella* and *Campylobacter* prevalence. A total of 636 caeca samples were collected; 376 from Western Australia and 260 from South Australia.

The concentration of *Salmonella* and *Campylobacter* in the caeca was also quantified for positive samples. Caecal samples were collected at the evisceration step in the poultry processing plants and relevant information recorded in Form B: Sample collection at processing facilities (Attachment 3).

The procedure used to collect the caeca was as follows:

1. After viscera were removed from the carcass, intact viscera were selected using clean gloves.
2. Caeca from intestines at junction with hindgut were removed.
3. Caeca was placed into a clean plastic bag, sealed and labelled.
4. Sample was transported chilled to the laboratory.

The procedure for preparing the caeca samples at the laboratory was as follows:

1. Upon receipt of the caeca into the laboratory, the contents of the caeca were milked into a suitable sterile container and the mass recorded.

2. Half of the contents of the container were placed into another sterile container to provide two aliquots for testing, one for *Campylobacter* and one for *Salmonella*.

Preparation of caeca samples for *Campylobacter* testing

- a) Culture the caecal content directly onto plates of the agar media described in *Australian Standard AS 5013.6 – 2004 “Food Microbiology Method 6: Examination for specific organisms – Campylobacter”*.
- b) Perform serial dilutions on the caecal content as per AS1766.1.2 - 1991 Food Microbiology – “General procedures and techniques – preparation of dilutions” and spread onto one of the types of plates described in AS 5013.6 – 2004 “Food Microbiology Method 6: Examination for specific organisms – *Campylobacter*” as per AS1766.1.4-1991 “Food Microbiology – General procedures and techniques – Colony counts –surface spread method”.
- c) Incubate the plates as in *AS 5013.6 – 2004 “Food Microbiology Method 6: Examination for specific organisms – Campylobacter”* and proceed with confirmation of typical colonies as they are identified.
- d) Using the dilution and colony confirmation information calculate the concentration of *Campylobacter* per gram of caecal content.

Preparation of caeca samples for *Salmonella* testing

- a) Examine for *Salmonella* as per the “*AS 5013.10-2004: Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Salmonella spp*”.
- b) After making the initial dilution in Buffered Peptone Water, remove sufficient amount of this dilution to carry out a 3 tube Most Probable Number (MPN) enumeration as described in Attachment 2: Method of analysis for *Salmonella* and *Campylobacter*.

For further detail on the methods used for the confirmation and quantification of *Salmonella* and *Campylobacter*, see Attachment 2: Method of analysis for *Salmonella* and *Campylobacter*.

6.3 Post processing

To determine the prevalence and concentration of *Salmonella* and *Campylobacter*, post processing, rinse samples from chicken carcasses were taken from processing plants, following the chilling step. Four jurisdictions participated in this component of the survey – WA, SA NSW and Qld. A proportional sampling approach was utilised within each state based on throughput at each plant and sample numbers based on the expected prevalence of *Salmonella* and *Campylobacter* in that state. A total of 1112 carcass rinse samples were collected - 375 from Western Australia, 341 from South Australia, 246 from New South Wales and 150 from Queensland.

Rinse samples were also tested for Total Viable Counts (TVC) and *Escherichia coli*.

The procedure for collecting the rinse samples was as follows.

1. Samples were collected at the processing facility immediately after the active chilling process, with the weight (in grams) recorded either at the facility or at the laboratory.
2. Samples were placed into a sterile plastic bag and the temperature recorded.
3. Relevant information was recorded in Form B: Sample collection at processing facilities (Attachment 3).
4. Samples were transported to the laboratory chilled.
5. On arrival at the laboratory, temperature of samples was measured.

Samples were prepared based on the rinse technique in the Australian Standard AS 5013.2004: *Preparation of test samples for microbiological examination – Poultry and poultry products*,⁹ either at the processing facility or at the receiving laboratory.

The procedure used was:

1. Add 500 ml of 0.1% buffered peptone water to the carcass in plastic bag.
2. Remove approximately half the air from the bag by massaging around the sample from the closed end to the open end. Tie the open end.
3. Shake and massage the sample vigorously for 2 minutes ensuring thorough rinsing of the abdominal cavity.
4. Release the rinse fluid into a sample container by cutting of the corner of the bag with scissors and allowing the fluid to drain into the container. This rinse fluid is the first dilution for the sample analysis.

Rinse samples were tested for:

- *Salmonella* using AS 5013.10-2004: *Food microbiology – Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella spp.*
- *Campylobacter* using AS 5013.6 – 2004 “*Food Microbiology Method 6: Examination for specific organisms – Campylobacter*”.

Total Viable Count by:

1. Preparing 1:10 dilutions in 0.1% peptone diluent;
2. Inoculating 1 ml from each dilution onto Petrifilm Aerobic Plate Count Plates (3M);
3. Incubating at 25°C for 4 days; and
4. Counting and identifying colonies as per manufacturer’s instructions.

E. coli by:

1. Preparing 1:10 dilutions in 0.1% peptone diluent;
2. Inoculating 1 ml from each dilution onto Petrifilm *E. coli* Plate Count Plates (3M);
3. Incubating at 37°C for 2 days; and

⁹ In NSW the AS1766 series was used (AS1766.0-1995 Food Microbiology – General Introduction and List of Methods).

4. Counting and identifying colonies as per manufacturers' instructions.

For further detail on the methods used for the confirmation and quantification of *Salmonella* and *Campylobacter*, see Attachment 2: Method of analysis for *Salmonella* and *Campylobacter*.

7. Method of Analysis

The pre-processing (cecal contents) data for *Campylobacter* and *Salmonella* counts were converted to \log_{10} cfu/g.

The post processing (rinse) data for TVC, *E. coli* and *Campylobacter* counts were converted to \log_{10} cfu/cm² and *Salmonella* was converted to \log_{10} MPN/cm², according to the formulae for carcasses in the Australian Standard, AS 5013.20–2004 *Method 20: Preparation of test samples for microbiological examination-Poultry and poultry products*.

For the carcass rinse results for *Campylobacter*, the results were also calculated on a per carcass basis to enable comparisons with overseas data. To enable the conversions, the carcasses were all assumed to have an average weight of 1.78 kg, which was the average dressed weight of meat chickens at the end of primary processing in 2007/08¹⁰ (ACMF, 2009). The calculations were performed multiplying the *Campylobacter* concentration (cfu/g) by the average carcass weight of 1780 g. The result (cfu/carcass) was then converted to the \log_{10} scale.

Mean concentrations and standard errors are presented in summary tables for each state. Standard errors are calculated from pooled variance estimates. Approximate standard deviations could be obtained by multiplying the standard error by the square root of the number of positives.

A censored regression (Helsel, 2005) was performed for *Campylobacter* and *Salmonella* counts (per square centimetre) to obtain censored means for each state. By censored we refer to data where the actual amount of *Salmonella* (or *Campylobacter*) is undetected or unknown in positive samples because it is less or greater than the limit of detection. Consequently, all *Campylobacter* (or *Salmonella*) samples were assumed to be positive, that is, samples where *Campylobacter* (or *Salmonella*) were not detected were still assumed to be positive, but with concentrations below the lower limit of detection.

¹⁰ Data on the chicken meat industry is compiled by the Australian Bureau of Agricultural and Resources Economics and a summary is provided on the Australian Chicken Meat Federation website, www.chicken.org.au under 'Industry Facts and Figures'.

8. Results

Results are presented below for the three components of the survey - on farm (Section 8.1), just prior to processing (Section 8.2) and at the end of primary processing (Section 8.3). Samples that were positive for *Salmonella* were typed to determine the serovars. The results of this testing are presented in Section 8.4.

8.1 On-farm (faecal samples)

Pooled faecal samples were collected from 39 farms in Western Australia as an indicator of flock prevalence on farm from October 2007 until March 2008. Samples were collected from 233 individual sheds on the farms. Each farm had between four and twelve sheds sampled and generally each shed had between 20 to 30 thousand chickens per shed. All samples were taken before first pick up and pooled to collect 250gm. Samples were taken before the first pick up so that all the results could be compared.

Additional information was collected at the time of sampling in relation to the age of the flock, whether the flock had been depopulated prior to sample collection¹¹ and what water source was used (see Attachment 1). This information has not been included as it was incomplete.

Table 1 summarises the results of the testing. Samples that were positive for *Salmonella* were typed to determine the serovars, and the results have been grouped into those that were Sofia and those that were non-Sofia. Sofia is considered to have low virulence to humans (i.e. rarely implicated in cases of human salmonellosis).

Table 1: Summary statistics for total *Salmonella* spp. flock/shed prevalence and *Campylobacter* spp. flock/shed prevalence for faecal samples in WA

Pathogen	# +’ve samples (n=233)	%	95%CI	# +’ve farms (n=39)	%	95% CI
<i>Salmonella</i>	109	46.8	(40.2, 53.4)	33	84.6	(69.5, 94.1)
Non-Sofia	109	46.8	(40.2, 53.4)	33	84.6	(69.5, 94.1)
Sofia	2	0.9	(0.1, 3.1)	1	2.6	(0, 13.5)
<i>Campylobacter</i>	150	64.4	(57.9,70.5)	28	71.8	(55.1,85)

Key Observations

- Of the 233 sheds tested, 46.8% were positive for *Salmonella*, comprising of 46.8% positive for non-Sofia serovars and 0.9% of samples positive for Sofia. The two samples that were positive for Sofia were also positive for Give (see Attachment 4 for results of serovar testing).
- 64.4% of the sheds were positive for *Campylobacter*.
- In 21 sheds (9%), *Salmonella* and *Campylobacter* were not detected.
- Of the 39 farms sampled, 84.6% had at least one shed positive for *Salmonella* and 71.8% had at least one shed positive for *Campylobacter*.

¹¹ The method required that faecal droppings be collected from sheds just before the first pick up for consistency.

- In some farms *Salmonella* or *Campylobacter* were not detected (15.4% and 28%, respectively). However, there were no farms where neither *Salmonella* and *Campylobacter* were detected, with at least one shed testing positive for *Salmonella* or *Campylobacter* on every farm.

8.2 Prior to processing (caecal samples)

To determine the prevalence of *Salmonella* and *Campylobacter* in poultry entering processing facilities, the caecal contents of poultry were collected for testing. The caeca were isolated from the viscera, following the evisceration step during processing.

South Australia and Western Australia participated in this component of the survey with a total of 636 caecal samples collected. The concentrations of *Salmonella* and *Campylobacter* in the caeca were also quantified for positive samples. This quantification can give an indication of the frequency of ‘high-shedders’ (i.e. percentage of birds with high concentrations of the pathogens in their caeca).

In South Australia samples were collected from March-May 2007 and in Western Australia from October 2007 until March 2008.

Additional information was collected at the time of sampling in relation to crate time (how long poultry had been held in crates), the age of the flock, number of previous pick-ups from shed and last time poultry was fed (see Attachment 3).

Where recorded, crate time varied from 5 to 22 hours, the average age of the flocks tested was 31 days, with a range of 26-44 days. The majority of flocks tested had come from sheds that had not previously been depopulated. The last feed time varied from 4 to 10 hours.

8.2.1 *Salmonella* results (caecal samples)

Table 2a: Summary statistics for total *Salmonella* spp. prevalence and counts by State for caecal contents

State	Sample No	Total <i>Salmonella</i> spp.				
		# +’ve	%	95%CI	mean log* (SE) MPN/gm	Censored mean log (SE) MPN/gm
SA	260	66	25.4	(20.2, 31.1)	0.92 (0.09)	0.008 (0.19)
WA	376	15	4.0	(2.2, 6.5)	1.56 (0.21)	1.44 (0.38)
SA/WA	636	81	12.7	(10.2, 15.6)	1.02 (0.09)	0.14 (0.18)

* Mean and standard errors were calculated for positive samples only

Table 2b: Summary statistics for *Salmonella* (non-Sofia and Sofia) serovar prevalence and counts by state for caecal contents

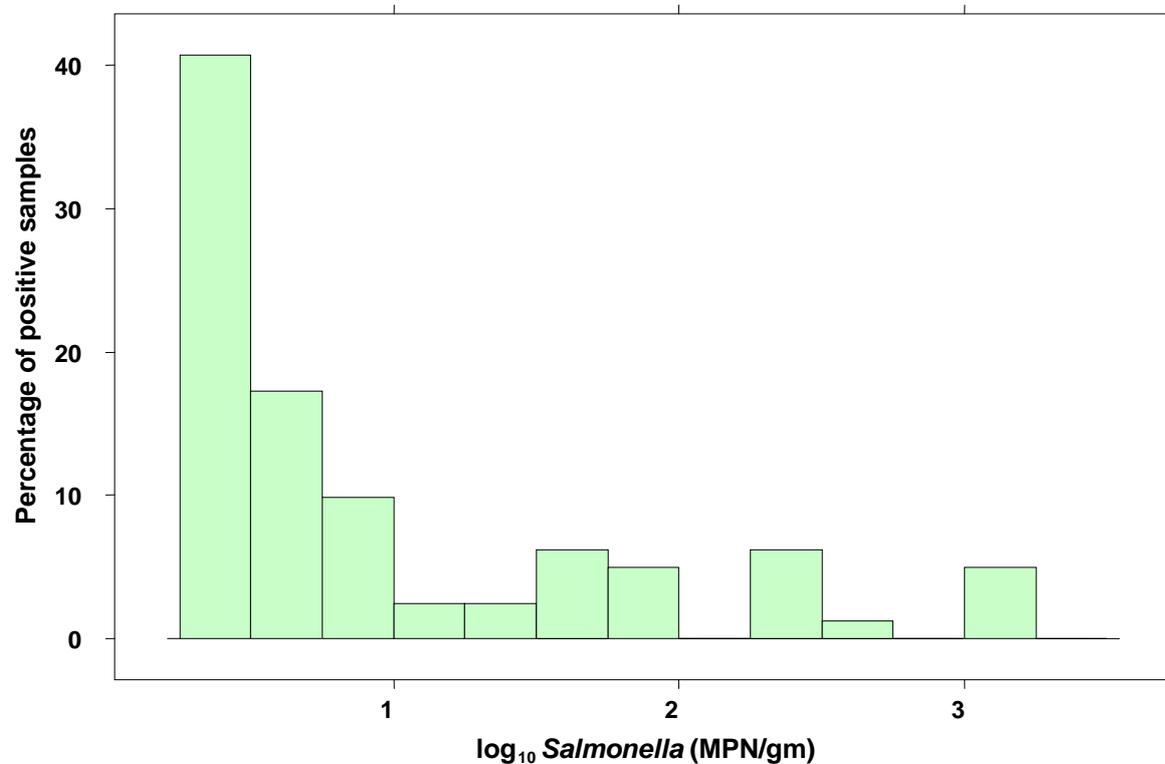
State	Sample No	Non-Sofia				Sofia			
		# +ve	%	95%CI	mean log* (SE)/gm	# +ve	%	95%CI	mean log* (SE)/gm
SA	260	33	12.7	(8.9, 17.4)	1.01 (0.14)	33	12.7	(8.9, 17.4)	0.85 (0.11)
WA	376	15	4.0	(2.0, 6.5)	1.56 (0.24)	0	0	(0, 0.9)	NA
SA/WA	636	48	7.5	(5.6, 9.9)	1.16 (0.13)	33	5.2	(3.6, 7.2)	0.85 (0.11)

* Mean and standard errors were calculated for positive samples only

Key Observations

- 12.7% of caecal samples were positive for *Salmonella* comprising of 7.5% positive for non-Sofia serovars and 5.2% positive for Sofia. Some samples had multiple serovars, see Section 8.4.
- *Salmonella* prevalence was 4% in WA and 25.4% in SA.
- The mean concentration of positive samples for the participating states was 1.02 log₁₀ MPN/gm and 0.14 log₁₀ MPN/gm using the censored approach.
- The mean concentration of positive *Salmonella* samples for the participating states was 0.92 log₁₀ MPN/gm in SA and 1.56 log₁₀ MPN/gm in WA. The higher mean concentration for WA is most likely a consequence of many of the positive SA samples having counts below the limit of detection (i.e. <3 MPN/gm).

Figure 1: Distribution of total *Salmonella* spp counts from caecal contents for WA and SA



8.2.2 *Campylobacter* results (caecal samples)

Table 3: Summary statistics for *Campylobacter* prevalence and counts by State from caecal contents

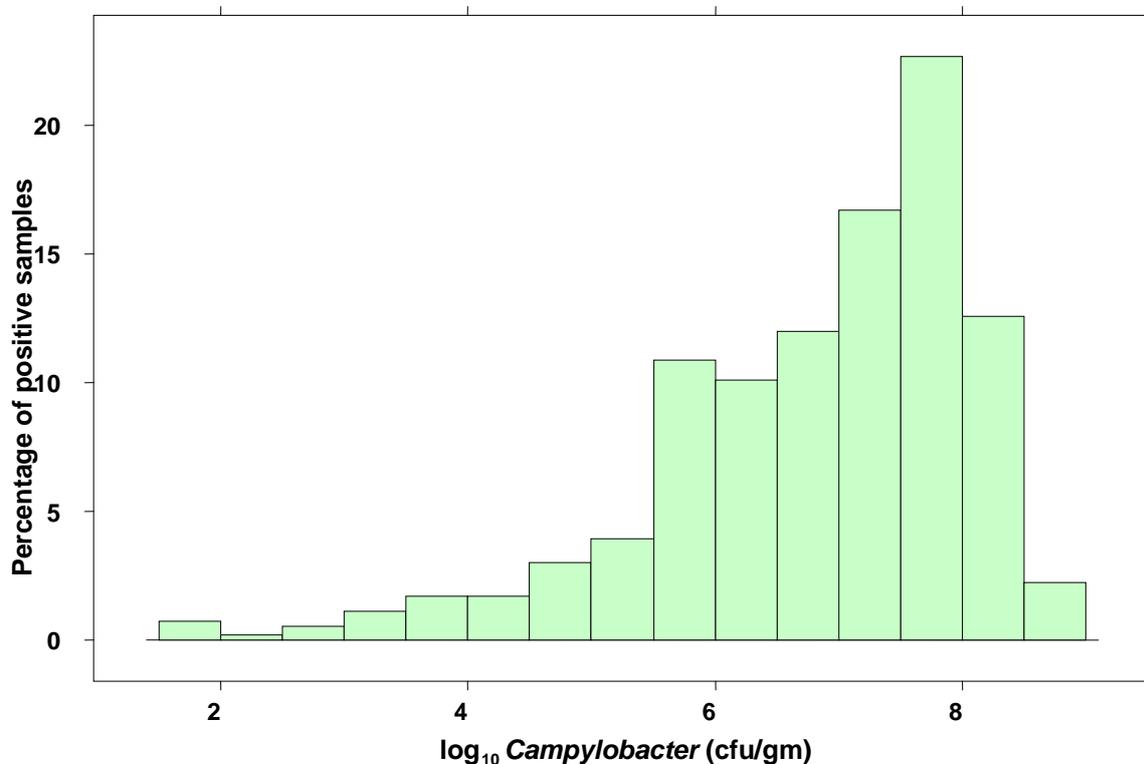
State	Sample No	<i>Campylobacter</i>				
		# +ve	%	95%CI	mean log* (SE) cfu/gm	Censored mean log (SE) cfu/gm
SA	260	217	83.5	(78.4, 87.8)	7.26 (0.08)	6.21 (0.16)
WA	376	317	84.3	(80.2, 87.8)	6.60 (0.07)	5.70 (0.13)
SA/WA	636	534	84.0	(80.9, 86.7)	6.87 (0.06)	5.91 (0.10)

* Mean and standard errors were calculated for positive samples only

Key Observations

- 84% of caecal samples were positive for *Campylobacter*.
- The prevalence of *Campylobacter* was similar in both states, 83.5% in SA and 84.3% in WA.
- The mean concentration of positive samples for the participating states was 6.87 log₁₀ cfu/gm and 5.91 log₁₀ cfu/gm after adjusting for samples below the limit of detection.
- *Campylobacter* (positive) counts were somewhat higher in SA compared to WA (mean difference 0.66).

Figure 2: Distribution of *Campylobacter* counts from caecal contents for WA and SA



8.3 Post processing (rinse samples)

In total, 1112 carcass rinse samples were collected from four states (NSW, QLD, SA and WA). Samples were collected at the end of processing, after the chilling step. Additional information was collected at the time of sampling in relation to the type of chilling system and the pH and chlorine concentration in the spin chiller at the time of sampling (see Attachment 3). Where recorded, the pH and chlorine concentrations were within acceptable limits - pH 5.0-7.5 and free chlorine 3.0-5.0ppm.

In some cases, the number of samples initially planned was not feasible for that state and so a reduced number of samples were collected or not all samples were tested. This was particularly the case for the testing of the samples for *E. coli* and TVC. The emphasis was placed on testing for *Salmonella* and *Campylobacter*. However, enough samples were collected and tested to have a high level of confidence in the data.

8.3.1 *Salmonella* results (post processing)

Table 4a: Summary statistics for total *Salmonella* spp. prevalence and counts from carcass rinse post spin chill

State	Sample No	Total <i>Salmonella</i> spp.				
		# +ve	%	95%CI	mean log* (SE) MPN/cm ²	Censored mean log (SE) MPN/cm ²
NSW	246	119	48.4	(42.0, 54.8)	-1.90 (0.03)	-2.69 (0.08)
Qld	150	66	44.0	(35.9, 52.3)	-1.98 (0.04)	-3.24 (0.15)
SA	341	176	51.6	(46.2, 57.0)	-2.05 (0.03)	-2.89 (0.08)
WA	375	47	12.5	(9.4, 16.3)	-2.03 (0.05)	-3.48 (0.13)
National	1112	408	36.7	(33.9, 39.6)	-1.99 (0.02)	-3.04 (0.08)

* Mean and standard errors were calculated for positive samples only from participating states

Table 4b: Summary statistics for *Salmonella* (non-Sofia and Sofia) serovar prevalence and counts by State from carcass rinse post spin chill

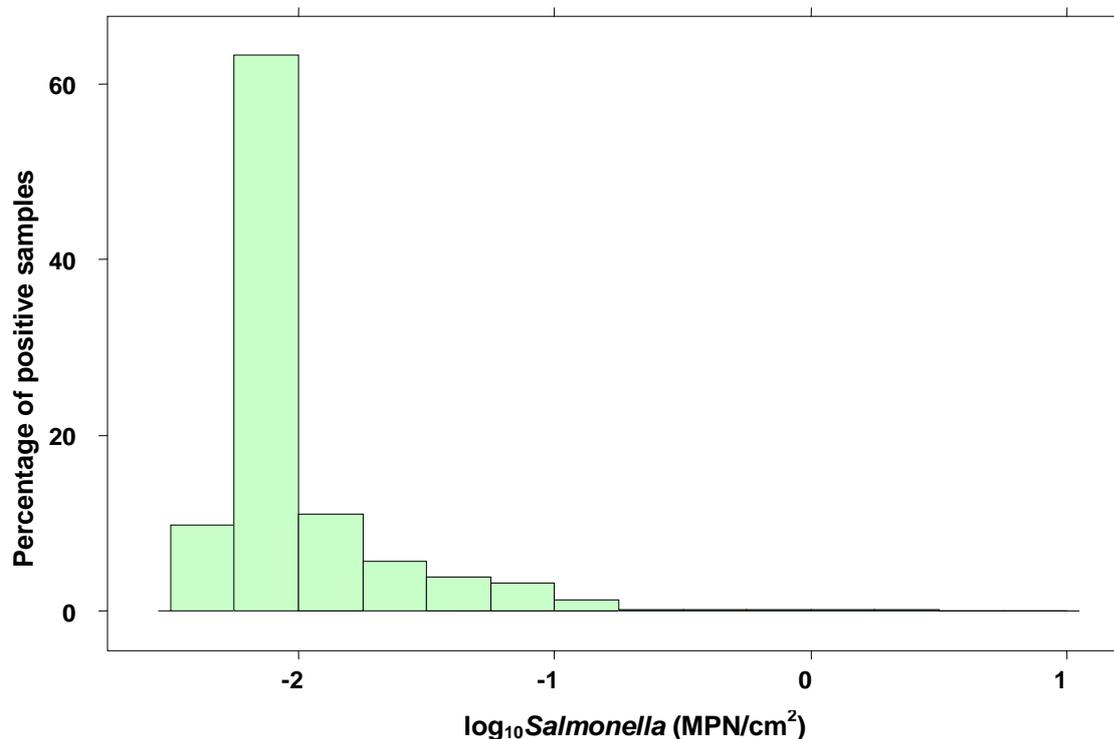
State	Sample No	Non-Sofia				Sofia			
		# +ve	%	95%CI	mean log* (SE)/cm ²	# +ve	%	95%CI	mean log* (SE)/cm ²
NSW	246	52	21.1	(16.2, 26.8)	-1.85 (0.05)	70	28.5	(22.9,34.5)	-1.93 (0.04)
Qld	150	10	6.7	(3.2, 11.9)	-1.97 (0.11)	56	37.3	(29.6, 45.6)	-1.98 (0.05)
SA	341	137	40.2	(34.9, 45.6)	-2.04 (0.03)	42	12.3	(9.0, 16.3)	-2.10 (0.06)
WA	375	47	12.5	(9.4, 16.3)	-2.03 (0.05)	0	0	(0, 1.0)	NA
National	1112	246	22.1	(19.7, 24.7)	-1.99 (0.02)	168	15.1	(13.1, 17.3)	-1.99 (0.03)

* Mean and standard errors were calculated for positive samples only from participating states

Key Observations

- 36.7% of rinse samples were positive for *Salmonella* comprised of 22.1% positive for non-Sofia *Salmonella* serovars and 15.1% positive for Sofia. Some samples had multiple serovars, see Section 7.4.
- The mean concentration for positive samples from the participating states was $-1.99 \log_{10}$ MPN/cm².
- The prevalence of *Salmonella* ranged from 12.5% in WA to 51.6% in SA.
- Mean log concentrations of positive samples of *Salmonella* from the participating states ranged from $-2.05 \log_{10}$ MPN/cm² in SA to $-1.90 \log_{10}$ MPN/cm² in NSW.

Figure 3: Distribution of total *Salmonella* spp. counts (positive samples only) from chicken rinse post spin chill across participating states (NSW, Qld, SA, WA)



8.3.2 *Campylobacter* results (post processing)

Table 5: Summary statistics for *Campylobacter* prevalence and counts from chicken rinse post spin chill

State	Sample No	<i>Campylobacter</i>					
		# + ⁺ ve	%	95% CI	mean log* (SE) cfu/cm ²	Censored mean log (SE) cfu/cm ²	Mean log* (SE) cfu/1.78kg bird
NSW	246	234	95.1	(91.6, 97.5)	0.73 (0.03)	0.48 (0.06)	4.07 (0.05)
Qld ⁺	150	96	64.0	(55.8, 71.7)	1.45 (0.05)	0.39 (0.11)	4.79 (0.14)
SA	333	269	80.8	(76.1, 84.9)	0.66 (0.03)	0.20 (0.05)	4.00 (0.05)
WA	375	332	88.5	(84.9, 91.6)	0.50 (0.03)	-0.05 (0.06)	3.84 (0.05)
National	1104	931	84.3	(82.0, 86.4)	0.70 (0.02)	0.18 (0.04)	4.04 (0.03)

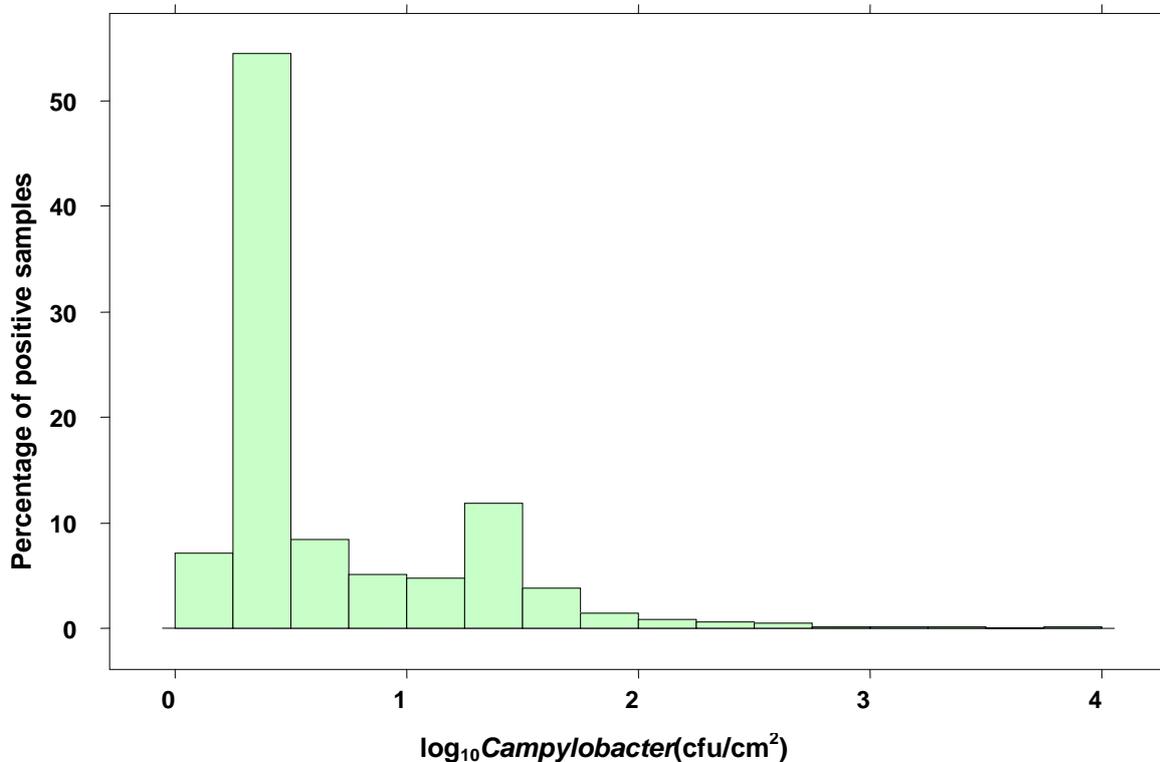
* Mean and standard errors were calculated for positive samples only from participating states

⁺ The limit of detection in Qld was 100 cfu/ml for the quantitative test. In other states the limit of detection was 10 cfu/ml.

Key observations

- 84.3% of rinse samples were positive for *Campylobacter*.
- The prevalence of *Campylobacter* ranged from 64% in Qld to 95.1% in NSW.
- The mean concentration of positive samples from the participating states was 0.70 log₁₀ cfu/cm² or 4.04 log₁₀ cfu/carcass.
- Qld *Campylobacter* counts are on average higher than other states as they only detected counts >100 cfu/ml.
- Mean log concentrations for positive *Campylobacter* samples from the participating states ranged from 0.50 log₁₀ cfu/cm² (3.84 log₁₀ cfu/carcass) in WA to 1.45 log₁₀ cfu/cm² (4.79 log₁₀ cfu/carcass) in Qld.

Figure 4: Distribution of *Campylobacter* counts from chicken rinse post spin chill across participating states (NSW, Qld, SA, WA)



8.3.3 Total Viable Count and *E. coli* results

The majority of rinse samples were tested for TVC and *E. coli* as an indication of how hygienically the process facilities were operating. Unsatisfactory results can indicate that a step in the poultry processing is out of control and corrective action needs to be taken.

Table 6 below categorises counts for TVC and *E. coli* from excellent to poor. These categories were used to assist with the interpretation of the data.

Table 6: Performance categories for TVC and *E. coli* on chicken meat products

Category Descriptor	TVC/cm ²	<i>E. coli</i> /cm ²
Excellent	<5,000	<10
Good	5,000-50,000	10-100
Acceptable	50,000-500,000	100-1,000
Marginal (for TVC) Action required (for <i>E. coli</i>)	500,000-1,500,000	>1,000
Poor ¹²	>1,500,000	

Source: Sumner *et. al.* (2004a)

8.3.3.1 Total Viable Count results

Of the 1112 rinse samples collected, post processing, 794 were tested for TVC in NSW, SA and WA. None were tested in Queensland. For interpretation of the category descriptors (excellent to poor), refer to Table 6.

Table 7: Total Viable Counts from chicken rinse post spin chill and conformance with performance categories

State	Sample No	Category Number (%)					Mean log TVC cfu/cm ² (SE)
		Exc*	Good	Accept*	Marg*	Poor	
NSW	80	68 (85.0)	12 (15.0)	0	0	0	2.52 (0.09)
SA	340	290 (85.3)	48 (14.1)	2 (0.6)	0	0	2.74 (0.04)
WA	374	374 (100.0)	0	0	0	0	1.10 (0.04)
National	794	732 (92.2)	60 (7.5)	2 (0.3)	0	0	1.95 (0.04)

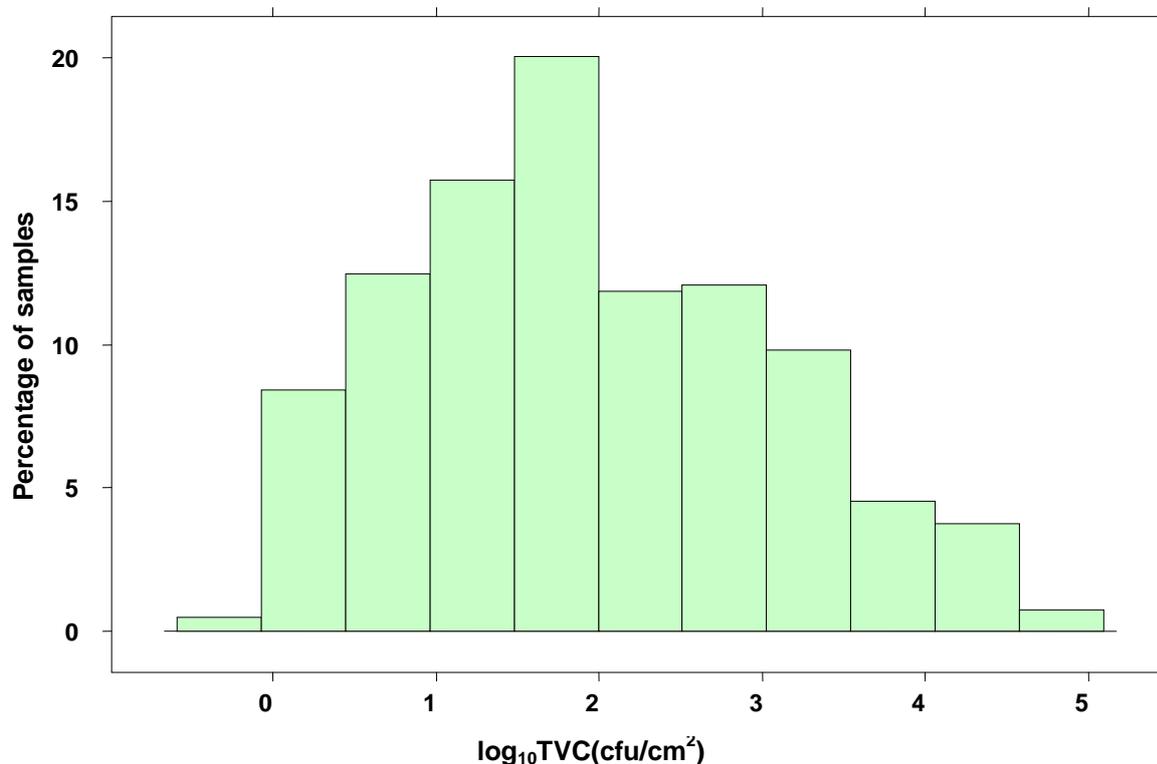
*Exc=Excellent, Accept=Acceptable, Marg=Marginal

Key observations:

- 99.7% of samples were either Excellent or Good.
- The remaining samples (0.3%) were classified as Acceptable.
- No samples were classified as either Marginal or Poor.
- Mean concentrations of TVC ranged from 1.10 log₁₀ cfu/cm² in WA to 2.74 log₁₀ cfu/cm² in SA.

¹² This category has been added.

Figure 5: Distribution of TVC from chicken rinse post spin chill across participating states (NSW, SA, WA)



8.3.3.2 *E. coli* results

Of the 1112 rinse samples, 791 in total were tested for *E. coli* in NSW, SA and WA. None were tested in Queensland. For interpretation of the category descriptors (excellent to poor), refer to Table 6.

Table 8: *E. coli* (for positive samples only) from chicken rinse post spin chill and conformance with performance categories

State	Sample No	# of positive samples (%)	Category Number (%)				Mean log [*] count cfu/cm ² (SE)
			Exc ⁺	Good	Accept ⁺	Action Required	
NSW	80	77 (96.3)	57 (74.0)	17 (22.1)	3 (3.9)	0	0.55 (0.08)
SA	337	311 (92.3)	163 (52.4)	117 (37.6)	31 (10.0)	0	0.93 (0.04)
WA	374	292 (78.1)	276 (94.5)	13 (4.5)	3 (1.0)	0	-0.03 (0.04)
National	791	680 (86.0)	496 (72.9)	147 (21.6)	37 (5.4)	0	0.48 (0.03)

⁺Exc=Excellent, Accept=Acceptable

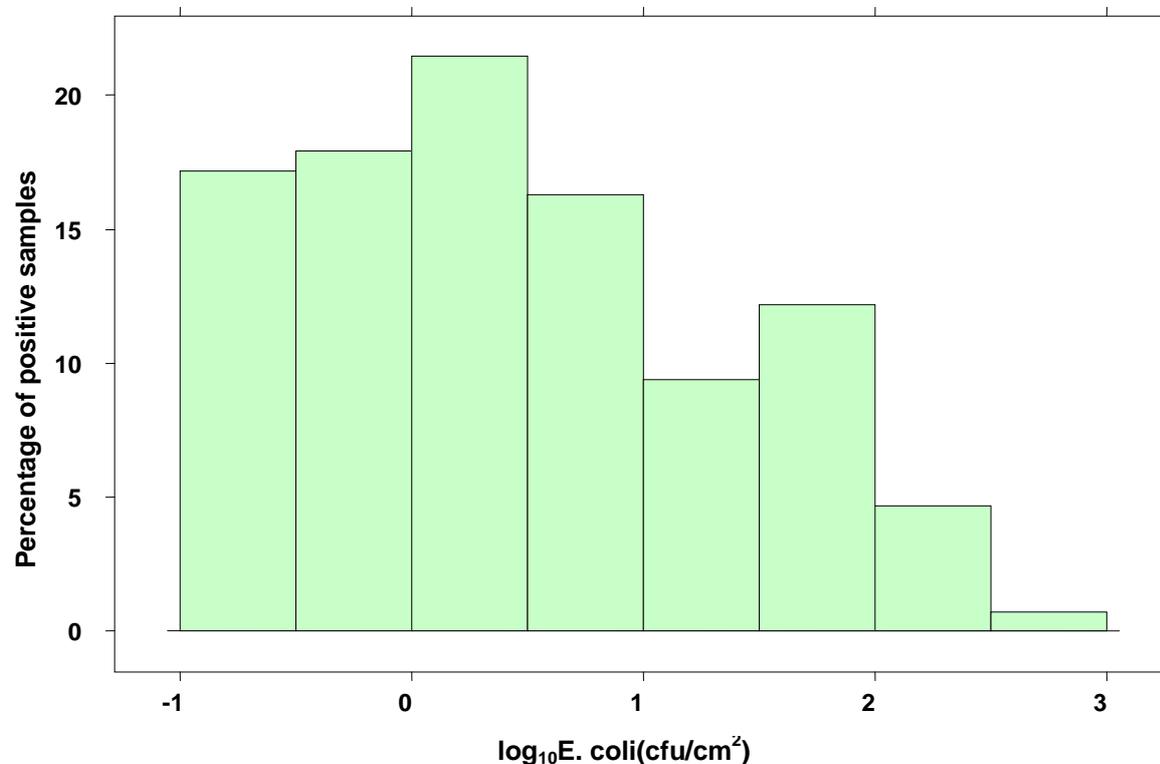
^{*}Mean and standard errors were calculated for positive samples only from participating states

Key observations

- 94.5% of samples were classified as Excellent or Good and the remainder were all classified as Acceptable.
- No samples were classified in the Action Required category.
- 86% of samples tested were positive for *E. coli*, with a mean concentration of 0.48 log₁₀ cfu/cm².

- The prevalence of *E. coli* ranged from 78.1% in WA to 96.3% in NSW.
- Mean concentrations ranged from $-0.03 \log_{10} \text{ cfu/cm}^2$ in WA to $0.93 \log_{10} \text{ cfu/cm}^2$ in SA.

Figure 6: Distribution of *E. coli* (positive samples only) from chicken rinse post spin chill across participating states (NSW, SA, WA)



8.4 *Salmonella* serovar testing

Samples that were positive for *Salmonella* were typed to determine the serovars. The results of this typing are presented in a table in Attachment 4. Multiple *Salmonella* serovars were isolated from some samples.

All phage typing for Typhimurium was conducted using the Colindale International Typing Scheme in NSW and SA with the exception of WA, where samples were tested by Pulsed field gel electrophoresis (PFGE). This testing does not allow for phage typing of Typhimurium serovars and therefore they are reported as Typhimurium only.

Key Observations

- The most common serovar isolated was Sofia. However, this serovar was uncommon in the positive samples from WA. Of the 171 positive *Salmonella* samples from the three components of the survey in WA, only two samples tested positive for Sofia.
- Following Sofia, the most common serovars were Typhimurium, Infantis and Mbandaka.

9. Discussion

9.1 On-farm (faecal samples)

9.1.1 Discussion of results (faecal samples)

Pooled faecal samples were collected from 39 farms in Western Australia as an indicator of flock prevalence. The pooling of the samples means that there may have been a mix of positive and negative faeces¹³. Therefore the results give a general indication as to the degree of infection within a shed and not individual bird prevalence.

Of the 233 sheds sampled, 46.8% were positive for *Salmonella* comprising of 46.8% positive for non-Sofia serovars and 0.9% of samples positive for Sofia¹⁴. For *Campylobacter*, 64.4% of the sheds were positive. In 21 sheds (9%), *Salmonella* and *Campylobacter* were not detected. In some farms *Salmonella* or *Campylobacter* were not detected (15.4% and 28%, respectively) but there were no farms where both pathogens were not detected, with at least one shed testing positive for *Salmonella* or *Campylobacter* on every farm.

9.1.2 Comparison with overseas surveys (faecal samples)

Table 9 below summarises results from recent overseas studies on prevalence of *Salmonella* and *Campylobacter* in broiler flocks where prevalence was estimated by pooling faecal or caecal samples. Caution needs to be taken when comparing studies, due to the differences in survey design, methodology and sample sizes and therefore the comparison is somewhat rudimentary.

The studies have been split according to whether they were baseline surveys or whether they were surveys conducted in countries that have specific interventions in place to lower the prevalence of these pathogens. Currently in Australia there are no specific measures to lower the prevalence of *Campylobacter* and *Salmonella* in poultry flocks. The majority of poultry growers comply with an industry manual, *National Biosecurity Manual for Contract Meat Chicken Farming*. This manual was developed by the Australian Chicken Meat Federation in 2002 and forms part of or is directly or indirectly referred to in most contracts governing the farming of chicken on behalf of chicken processors. This manual specifies the biosecurity measures necessary to prevent the introduction of infectious diseases to poultry and the spread of disease from an infected area to an uninfected area. While this includes *Campylobacter* and *Salmonella*, these pathogens have not been specifically targeted.

There were two studies that were baseline surveys, one in Canada and one in Ireland. If the results from this survey are compared with these, the prevalence of *Campylobacter* was higher (64.4% compared to 35% in Canada) and for *Salmonella*, very similar to Canada (46.8% compared to 50% in Canada) but higher than Ireland where the prevalence was 27%.

¹³ It is not known how many bird droppings went into each pooled sample and this may affect the prevalence.

¹⁴ The two samples that were positive for Sofia were also positive for Give.

In the Netherlands, a study reported lower prevalences of *Campylobacter* and/or *Salmonella*. However, there were specific interventions in place to control these pathogens.

In the Netherlands the *Salmonella* flock prevalence dropped from 20% in 1999 to 11% in 2002. During this period, the *Campylobacter* flock prevalence remained fairly stable at 20%. In 1997, the Dutch Products Boards for Livestock, Meat and Eggs implemented monitoring and control programs to reduce *Salmonella* and *Campylobacter* contamination of poultry meat. These programs include, amongst others, microbiological examination of flocks at each stage of the production chain, application of strict hygiene measures throughout the production chain and a logistic slaughtering procedure for broiler flocks (Van de Giessen et al, 2006).

Table 9: Summary of recent overseas studies on prevalence of *Salmonella* and *Campylobacter* in broiler flocks

Country	<i>Salmonella</i> flock prevalence ¹⁵	<i>Campylobacter</i> flock prevalence	Comments	Reference
Baseline surveys				
Western Australia (this survey)	46.8% (95% CI: 40.2, 53.4)	64.4% (95% CI: 57.9, 70.5)	233 sheds sampled on 39 farms. One pooled sample taken from each shed (~250g).	This survey.
Canada (Quebec)	50% (95% CI: 37, 64)	35% (95% CI: 22, 49)	Flock status was evaluated by culturing pooled caecal contents from about 30 birds per flock in 2003/04. Sample size was 81 flocks.	Arsenault et al, (2007a)
Ireland	27%		Baseline study conducted in 2006 in a total of 362 broiler flocks, representing 80% of total broiler population. Samples taken within last 3 weeks prior to depopulation. A total of five pairs of boot swabs collected per flock.	Gutierrez M et al (2009)
Specific interventions in place				
Netherlands	From ~20% in 1999 to ~11% in 2002	From 1999-2002, prevalence roughly averaged around 20%	Results of ongoing surveillance of broiler flocks in period 1999-2002 – 100-200 flocks sampled annually. Fresh faecal samples were randomly collected and pooled. In 1997, monitoring and control programs implemented to reduce <i>Salmonella</i> and <i>Campylobacter</i> contamination of poultry meat.	Van de Giessen et al (2006)

¹⁵ The *Salmonella* prevalences indicated in the table are for total *Salmonella*.

9.2 Prior to processing (caecal samples)

9.2.1 Discussion of results (caecal samples)

To determine the prevalence of *Salmonella* and *Campylobacter* in poultry entering processing facilities, the caecal contents of poultry were collected for testing in South Australia and Western Australia.

Overall, 12.7% of samples tested positive for *Salmonella*, with 7.5% being positive for non-Sofia serovars. In WA, 4% of samples were positive for non-Sofia serovars and in SA, 12.7% were positive. The mean concentration of *Salmonella* was low, 1.02 log₁₀ MPN/gm. For *Campylobacter*, the average prevalence was 84% and the mean concentration was 6.87 log₁₀ cfu/gm. For the two states the percentages were similar for *Campylobacter*, 83.5% (SA) and 84.3% (WA).

Direct comparisons with the results in Section 9.1.1 are not possible as one was testing the flock prevalence, whereas the caecal testing was estimating the bird prevalence across flocks. Within flock prevalence is often variable due to many factors such as the virulence of the *Salmonella* strain and immune status of birds within the flock (FAO/WHO, 2002). Only Western Australia participated in both components in this survey. For the pooled faecal samples, 46.8% tested positive for *Salmonella*, whereas only 4% of the caecal samples were positive for *Salmonella*. This may be explained by the fact that the pooled faecal samples can contain a mix of positive and negative faeces but when tested, a positive result is recorded.

The *Campylobacter* results are more consistent with expectations. For the pooled faecal samples, 64.4% were positive and for the caecal samples 84%. A higher percentage would be expected in the caecal samples as the birds tested were older. *Campylobacter* infections tend to increase with the age of the birds and once *Campylobacter* has colonised birds in a growing house, all birds in the flock become contaminated within a few days (Berndtson et al, 1996).

9.2.2 Comparison with overseas surveys (caecal samples)

A study in Iceland tested poultry just prior to processing for *Campylobacter* prevalence by testing the contents of the caeca (Barrios et al, 2006). Between May 2001 and Dec 2003, 1091 broiler flocks were tested by pooling caecal samples from four pooled samples of ten from each flock. The flock prevalence was low at 15% (95% CI: 13.3, 17.7), which is comparable to that of other northern European countries (Barrios et al, 2006). The low prevalence of *Campylobacter* found in this survey is also likely to be as a result of an official policy of testing flocks prior to slaughter and requiring that meat derived from positive flocks be sold frozen. Positive flocks are also processed either at the end of the processing day or week. The profit derived by the poultry industry is greatly reduced when *Campylobacter*-positive flocks are frozen. Consequently the industry is highly motivated to identify cost-effective interventions to control this pathogen by enhancing farm biosecurity measures (Stern et al, 2005).

9.3 Post processing (rinse samples)

9.3.1 *Salmonella* and *Campylobacter* results

9.3.1.1 Discussion of *Salmonella* and *Campylobacter* results (rinse samples)

To determine the prevalence and concentration of *Salmonella* and *Campylobacter*, post processing, rinse samples from carcasses were taken from processing plants, following the chilling step. A total of 1112 carcass rinse samples were collected from four states (NSW, Qld, SA and WA). In South Australia samples were collected from March – May 2007, in Western Australia, samples were collected from October 2007 until March 2008, in New South Wales from March – July 2008 and in Queensland from April – August 2008. Positive *Salmonella* and *Campylobacter* samples were enumerated to estimate prevalence.

For *Salmonella*, 36.7% of rinse samples were positive with 22.1% being positive for non-Sofia serovars. The results for total *Salmonella* were similar between the states (NSW 48.4%, Qld 44.0%, SA 51.6%), with the exception of Western Australia, which was much lower at 12.5%. However, this is likely to be because Sofia serovars were not detected in the samples from Western Australia. If the percentage of samples are compared for non-Sofia serovars, Qld had the lowest prevalence at 6.7%, followed by WA at 12.5%, NSW 21.1% and SA at 40.2%. The *Salmonella* concentrations on positive samples was low, on average $-1.99 \log_{10}$ MPN/cm² or about 1 per 100cm².

For *Campylobacter*, 84.3% of rinse samples were positive. The *Campylobacter* results were similar across the states, though they were lower in Queensland and highest in New South Wales. The results were 64.0% in Queensland, 80.8% in South Australia, 88.5% in Western Australia and 95.1% in New South Wales. While the samples in the states were taken at different times of the year, there is no correlation between the results and peak notification times for *Campylobacteriosis*. Notifications of *Campylobacteriosis* in Australia peak in the months of October, November and sometimes December (FSANZ, 2005). However, NSW, which recorded the highest results, sampled from March-July 2008.

In Western Australia and New South Wales, the *Campylobacter* concentrations of positive samples was higher than for *Salmonella*, on average, $0.70 \log_{10}$ cfu/cm² or ~ 500 cfu/100cm². In Queensland, where only counts >100 cfu/ml were recorded, the mean concentration was $1.45 \log_{10}$ cfu/cm² or ~ 2818 cfu/100cm². This approximate 1 log increase is consistent with the 1 log difference in limit of detection.

The results from the samples taken at the end of primary processing are similar to the results from the retail baseline microbiological survey carried out in 2005/2006 in South Australia and New South Wales. Table 11 below compares the prevalence and concentrations between the two surveys.

Table 11 – Comparison of results from previous retail baseline survey and this survey

Survey	Prevalence		Concentration	
	<i>Salmonella</i> (%)	<i>Campylobacter</i> (%)	<i>Salmonella</i> (log ₁₀ MPN/cm ²)	<i>Campylobacter</i> (log ₁₀ cfu/cm ²)
This survey	36.7	84.3	-1.99	0.70
Retail survey ¹⁶	43.3	90.0	-1.42 to -1.6 ¹⁷	0.78 to 0.87 ¹⁸

This comparison indicates that the prevalence and concentration of *Salmonella* and *Campylobacter* on chicken carcasses post processing in this survey, was similar to the prevalence and concentration of these two pathogens found on raw chicken in the retail survey.

9.3.1.2 Comparison with overseas surveys (rinse samples)

Table 12 provides a summary of recent overseas studies on prevalence and concentration of *Salmonella* and *Campylobacter* in broiler carcasses. In the table, the studies are split into two categories, those that were baseline surveys (similar to this one) and those which were undertaken in countries where specific interventions are in place to lower *Salmonella* and *Campylobacter* levels and concentration, post processing. This comparison is rudimentary as direct comparisons are not possible due to differences in survey design, methodology and sample sizes.

In Australia, businesses that process poultry must meet the requirements of an Australian Standard, AS 4465-2005 *Construction of Premises and Hygienic Production of Poultry Meat for Human Consumption*. This Standard requires poultry processors to develop and implement HACCP¹⁹ programs and also includes specific requirements relating to the design and construction of the premises, the processing of poultry, health and hygiene requirements and cleaning and sanitising (ANZFRMC, 2006).

The requirement for poultry processors to have HACCP program in place has been mandatory since late 1997 (Sumner et al, 2004b). The Standard requires microbiological testing to verify process control. However, the recommended test is Total Viable Count and not for specific pathogens. While the need to minimise the contamination of poultry with *Salmonella* is certainly recognised, it is not specifically targeted, and *Campylobacter* even less so.

¹⁶ Pointon et al, 2008.

¹⁷ The *Salmonella* mean count was -1.42 log₁₀ MPN/cm² in New South Wales and -1.6 log₁₀ MPN/cm² in South Australia.

¹⁸ The *Campylobacter* mean count was 0.78 log₁₀ cfu/cm² in South Australia and 0.87 log₁₀ cfu/cm² in New South Wales.

¹⁹ The Hazard Analysis and Critical Control Point (HACCP) system ensures the safety of food by requiring potential food safety hazards to be controlled at every step of a food's production and to keep records to demonstrate this is occurring.

Sumner et al (2004b) examined whether the introduction of mandatory HACCP in poultry processing facilities in Australia in late 1997 had affected the prevalence of *Salmonella* or of salmonellosis. This evaluation found that the trend of notification rates for salmonellosis was generally rising over time from 31.9 cases per 100 000 in 1991 to 35.8 cases per 100 000 in 2001. Overall *Salmonella* prevalence on chicken carcasses had improved slightly from an annual incidence of 25-35% since 1981 to 28-29% in 2000 and 2001, when taking into account the fact that detection methods had improved markedly. The major difference was that *S. sofia* had become the dominant strain, comprising 39% of isolated serovars in 1981-1985 to 89% of serovars in 1991-1994. When compared to the results from this survey, overall prevalence of *Salmonella* is slightly higher at 36.7%. However, with the exception of WA, *S. sofia* is still the dominant strain.

When the results of this survey are compared with similar baseline surveys, there were similarities, particularly with a baseline study conducted in Canada. In this study, the prevalence of both *Salmonella* (37.5%) and *Campylobacter* (75%) were very similar to the results from this survey (*Salmonella* 36.7% and *Campylobacter*, 84.3%). This is also the case with a baseline study conducted in the US in 1994-1995, where *Campylobacter* prevalence was reported at 88.2%. The prevalence for *Campylobacter* was higher than a baseline level of 70% estimated for the UK in 1995, based on the available surveillance data (UKFSA, 2009a). This level was determined prior to the commencement of a *Campylobacter* reduction strategy. A baseline survey in Sweden found a much lower prevalence of *Campylobacter* at 15%. However, the colder conditions in Sweden may account for this.

As expected, the *Salmonella* and *Campylobacter* results from this survey are somewhat higher than those found in studies conducted after specific interventions strategies have been implemented. These include New Zealand, United States and Sweden. The UK also has a lower *Salmonella* prevalence, but unlike NZ, US and Sweden, the UK's *Campylobacter* reduction strategy has not yet been successful at lowering *Campylobacter* prevalence on chicken meat.

In NZ, the prevalence of *Campylobacter* on chicken carcasses in the second quarter of 2007 was 57%, which was just prior to New Zealand setting poultry processing targets for levels of *Campylobacter* on carcasses. By the second quarter of 2008, this prevalence had almost halved to 30.6% (NZFSA, Dec 2008). The levels of *Campylobacter* on the carcasses were 3.07 log₁₀cfu/carcass in the second quarter of 2007, dropping to 2.41 log₁₀cfu/carcass (NZFSA, Dec 2008). In Australia, the *Campylobacter* levels on the poultry carcasses were on average higher with a mean of 4.04 log₁₀cfu/carcass. This estimate was calculated assuming an average carcass weight of 1.78 kg, which in 06/07 was the average carcass weight in Australia (ACMF, 2009). This is similar to the average carcass weight in New Zealand, which for 07/08 was 1.73 kg (PIANZ, 2009). Therefore, the data are comparable between the two countries.

The reported incidence of *Campylobacteriosis* in 2002 was almost 3 times higher in New Zealand (326 cases/100,000 in 2004) (Lake, 2006) than Australia (117 cases/100,000 in 2004) (The OzFoodNet Working Group, 2005). To address the high rates of human *Campylobacteriosis* in New Zealand, the New Zealand Food Safety Authority (NZFSA) set an organisational performance target of 50% reduction in the reported annual incidence of foodborne *Campylobacteriosis* after five years (NZFSA, Dec 2008). Poultry meat was established as the primary exposure pathway in NZ and therefore a comprehensive strategy was formalised in 2006 to reduce *Campylobacter* levels in chicken meat. This is primarily being achieved by the setting of a *Campylobacter* performance target. Standard processors must sample three poultry carcasses per processing day and are required to achieve microbiological criteria of an 80th percentile of 1200 cfu/carcass (3.08 log₁₀ cfu/carcass) (NZFSA, Jan 2008). When results are higher than the criteria, the processor is required to take corrective action. There are five response levels, depending on how high the *Campylobacter* levels are - the higher the count, the more comprehensive the response. The lowest response is for the processor to review its procedures and the highest response is government intervention at the processing plant.

The NZ *Campylobacter* reduction strategy has seen cases of *Campylobacter* infection, caused by food, being reduced by 50 percent (NZFSA, 2009). The NZFSA has advised that these reductions have been predominantly achieved by processors improving their good hygienic practices during slaughter and dressing. The increased use of processing aids has also made a significant contribution. Further activities have included broiler growers improving control measures on farm²⁰, improvement in packaging and safe food messaging to food distributors, retailers and consumers.

In the US, over the past 10 years, the poultry industry has reduced *Campylobacter* spp. contamination of processed broilers by more than 10-fold – adherence to HACCP procedures within the plants playing a large role in this reduction. During this period, the human disease caused by *Campylobacter* has been substantially reduced (Stern, Pretanik (2006).

In Sweden, controls have been in place for *Salmonella* since 1961 (Wierup et al, 1995). They were introduced as a result of a large *Salmonella* epidemic in 1953 (Wierup et al, 1995). The overall aim of the Swedish *Salmonella* control program is that animals sent for slaughter shall be virtually free from *Salmonella*, which ensures that animal products for human consumption will be free from *Salmonella* (FAO/WHO, Jan 2002). With respect to poultry, four main factors are in place to control *Salmonella*-

1. The breeding pyramid is kept free from *Salmonella* – all grandparent animals are imported and all are quarantined and repeatedly tested
2. Feed is free from *Salmonella* – import control of feed raw materials, mandatory heat treatment of compound feeding stuffs for poultry and a HACCP-based *Salmonella* control program in the feed industry
3. High hygiene and biosecurity standards are in place, preventing introduction of *Salmonella*
4. Elimination of the flock is always carried out in case of *Salmonella* infection in poultry irrespective of serotype (FAO/WHO, Jan 2002).

²⁰ At the farm level, generic aspects of biosecurity have been improved but currently it is accepted that this only results in a limited reduction in the level of contamination of slaughtered birds.

In 2005, the UK Food Standards Agency set a strategic target of achieving a 50% reduction in the incidence of UK produced chicken testing positive for *Campylobacter* by 2010 (UK Food Standards Agency, 2009a). The baseline, against which this target was to be measured, was set at 70% based on the surveillance data available at the time (UKFSA, 2009a). A key part of the strategy to achieve the 50% reduction is the ‘Cleaner Farms, Better Flocks’ program which aims to improve hygiene measures on broiler farms and ensure that best practices are followed at all times (UK Food Standards Agency, 2009b).

The key messages are:

- keep livestock away from poultry houses
- only allow essential visitors onto the farm
- use dedicated boots for each poultry house
- eliminate vermin
- wash and sanitise hands before and after visiting the poultry shed

The UK’s *Campylobacter* prevalence of 65% (post intervention) is similar to the baseline level of 70%, indicating that the *Campylobacter* reduction strategy has not yet been successful in lowering the prevalence of this pathogen on chicken at retail. However, the levels are still lower than found in this survey for *Campylobacter* prevalence on broiler carcasses post processing (84.3%).

Table 12: Summary of overseas studies on prevalence and levels of *Salmonella* and *Campylobacter* post processing

Country	<i>Salmonella</i> prevalence ²¹	<i>Campylobacter</i> prevalence/levels	Comments	Reference
Baseline surveys				
Australia (this survey)	36.7% (95% CI: 33.9, 39.6)	84.3% (95% CI: 82.0,86.4)	Baseline survey. 1112 carcass rinse samples tested.	This survey
Canada	37.5%	75%	Baseline survey. In 2004-2005, 1234 poultry carcass rinse samples tested.	Bohaychuk et al (2009)
United States	20%	88.2%	1994-1995 baseline study, 1297 chicken carcasses sampled.	USDA, April 1996
United Kingdom		70%	Baseline level in 2005 based on surveillance data prior to introduction of <i>Campylobacter</i> reduction strategy.	(UKFSA, 2009a)
Sweden		15% The 10 th and 90 th percentiles of <i>Campylobacter</i> numbers per carcasses were 3.0 and 5.0 log cfu, respectively, and the maximum was 7.1 log cfu.	Baseline study of broiler chickens. 636 carcasses were collected from the ten largest slaughterhouses ²² .	Lindblad M et al (2006)
Specific interventions in place				
New Zealand		From 57% in quarter 2 of 2007 (mean of 3.07 log ₁₀ cfu/carcass) decreasing to 30.6% in quarter 2 of 2008 (mean of 2.41 log ₁₀ cfu/carcass)	Risk management strategy to reduce foodborne <i>Campylobacteriosis</i> by 50 percent after 5 years was formalised in 2006. Poultry processing targets set in 2008.	NZFSA (Dec 2008)
United States		26%, concentrations varied – mean of less than 10cfu/carcass in march to 390 cfu/carcass in October.	HACCP systems required to be implemented in processing plants. 4200 samples taken randomly from 10 participating poultry processors over a 13 month period.	Stern NJ, Pretanik S (2006)
United Kingdom	6.6% ²³	65.2%	Survey conducted between May 2007 and Sep 2008 on fresh chicken at retail sale. 3274 samples tested.	UKFSA (2009a)
Sweden	0% ²⁴		Baseline study of broiler chickens. 636 carcasses were collected from the ten largest slaughterhouses.	Lindblad M et al (2006)

²¹ The *Salmonella* prevalences in the table are for total *Salmonella*.

²² These 10 plants are responsible for 99.9% of the ~70 million broiler chickens slaughtered each year in Sweden.

²³ *Salmonella* was not part of the *Campylobacter* reduction strategy, as the prevalence of *Salmonella* in chicken meat in the UK has remained low. It was 5.7% in 2001 (UKFSA, 2009).

²⁴ None of the sampled carcasses were positive for *Salmonella*. Because of measures taken in the Swedish *Salmonella* control program, all Swedish red and white meat and eggs are virtually free from *Salmonella*.

9.3.2 Total Viable Count and *E. coli* results

Of the 1112 rinse samples taken, approximately 790 were tested for TVC and *E. coli*. These tests were undertaken to give an indication of how hygienically the process facilities were operating. Unsatisfactory results can indicate that a step in the poultry processing is out of control and corrective action needs to be taken. For TVC, almost 100% of the samples were considered excellent or good and none were marginal or poor. For *E. coli*, 94.5% of samples were classified as excellent or good, and the remainder were all acceptable.

While the results of the TVC and *E. coli* were all within acceptable criteria, the testing of these indicator organisms in poultry at the end of the processing is not a good indicator of the likelihood of the poultry being contaminated with *Salmonella* and *Campylobacter*. While TVC and *E. coli* counts may have been well within acceptable limits, the percentage of poultry likely to be contaminated with *Salmonella* and *Campylobacter* at the end of processing was high, particularly for *Campylobacter*. A similar study in Sweden estimating the prevalence and concentrations of pathogenic and indicator bacteria on Swedish broiler chickens reached the same conclusion – no correlation was found between numbers of any indicator bacteria and numbers of pathogenic bacteria (Lindblad et al, 2006). The indicator organisms tested were total aerobic organisms and *E. coli*.

9.4 *Salmonella* serovar testing

Samples that were positive for *Salmonella* were typed to determine the serovars. This typing enabled the *Salmonella* positive samples to be grouped according to whether they were Sofia or non-Sofia. This is important because, based on epidemiological evidence, Sofia is not normally associated with causing illness in humans whereas the other serovars, such as Typhimurium, are. The typing also provides an indication of the types of serovars present in chicken and which are the most common. This can provide a useful reference when investigating human infections with *Salmonella*.

The most common serovar isolated was Sofia. However, this serovar was uncommon in the positive samples from Western Australia. Of the 171 positive *Salmonella* samples from the three components of the survey in Western Australia, only two samples tested positive for Sofia and these were from samples that were also positive for Give. Following Sofia, the most common serovars were Typhimurium, Infantis and Mbandaka, which are associated with human illness.

10. Follow up action

This survey provides baseline data on the prevalence and concentration of *Salmonella* and *Campylobacter* at both the primary production and primary processing stages of the chicken meat supply chain. When the Standard for Poultry Meat has been implemented, a follow up survey needs to be conducted to evaluate whether the Standard has been effective in lowering the prevalence and concentration of *Salmonella* and *Campylobacter* in poultry meat. As per this survey, it should be an ISC coordinated national survey, preferably with all jurisdictions participating that have chicken meat farms and/or processors within their state.

11. Conclusions

This survey has provided baseline data on the prevalence and levels of *Salmonella* and *Campylobacter* on chicken meat at both the primary production and primary processing stages of the chicken meat supply chain. The results from farms in Western Australia indicate that poultry are being infected with *Salmonella* and *Campylobacter* on farm, with flock prevalence from pooled faecal samples being 46.8% for *Salmonella* and 64.4% for *Campylobacter*. The results from Western Australia and South Australia indicate that a large percentage of the live poultry entering processing plants are infected with *Campylobacter* (84%) and to a much lesser extent, *Salmonella* (12.7%). Results from samples taken from four states (NSW, Qld, SA and WA) at the end of primary processing, indicated that the prevalence of poultry contaminated with *Campylobacter* was 84%, consistent with the prevalence for the poultry entering the processing plants. However, the prevalence of *Salmonella* was higher at the end of processing (36.7% total *Salmonella*, of which 22.1% were positive for non-Sofia serovars).

The results from the samples taken at the end of primary processing are similar to the results from the retail baseline microbiological survey carried out in 2005/2006 in South Australia and New South Wales. The results from this survey showed that 43.3% of chicken samples tested were positive for *Salmonella* (12.8% being non-Sofia) and approximately 90% were positive for *Campylobacter coli/jejuni* (Pointon et al, 2008). The mean *Salmonella* and *Campylobacter* counts were also similar. This comparison indicates that *Salmonella* and *Campylobacter* contamination on chicken carcasses post processing, is likely to be carried through to the fresh chicken purchased by consumers.

When compared with results from similar baseline surveys overseas, the results from this survey were generally similar. Where countries have implemented specific strategies to lower *Salmonella* and *Campylobacter* in poultry, significant reductions have been reported. The exception to this is the UK, where a strategy to reduce *Campylobacter* prevalence on fresh chicken meat, has not yet been successful.

The data collected from this survey will be compared with those from a follow-up survey to be conducted after the new Standard for Poultry Meat has been fully implemented. This will assist in assessing the effectiveness of the new Standard for Poultry Meat in reducing the prevalence and concentration of *Salmonella* and *Campylobacter* in poultry and poultry meat.

12. References

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13. Attachments

1. Form A: Sample Collection On-Farm.
2. Method of analysis for *Salmonella* and *Campylobacter*.
3. Form B: Sample Collection at Processing Facilities.
4. *Salmonella* single and multiple serovar isolation profiles for positive samples

Attachment 1: Form A: Sample Collection On-Farm

FORM A: SAMPLE COLLECTION ON-FARM	
Baseline survey on the prevalence and levels of <i>Salmonella</i> and <i>Campylobacter</i> along the poultry meat supply chain	
<i>The following information is to be provided by all participating jurisdictions to allow analysis of data.</i>	
CONTACT DETAILS FOR PARTICIPATING JURISDICTION	
Name of sample collector:	Phone number:
E-mail address:	
Organization:	
INFORMATION ABOUT THE FARM BEING SAMPLED	
Facility being sampled:	
Has this facility previously been sampled? When?	
INFORMATION ABOUT THE SAMPLES	
Are there any circumstances whereby these samples could be compromised?	
Sample code	Sample code
1.	6.
2.	7.
3.	8.
4.	9.
5.	10.
What is the approximate age of the flock?	
Has the flock been depopulated ²⁵ ? How many times?	
What is the water source for the chickens?	

²⁵ In Australia, a percentage of chickens are harvested from most flocks on several occasions. Harvesting, also known as 'partial depopulation', 'thinning out', or 'multiple pick-up', may be done up to four times, depending on need for light or heavy birds. Thinning out sheds allows more space for the remaining birds and reduces the natural temperatures in the shed. The first harvest might occur as early as 30-35 days and the last at 55-60 days.

Attachment 2: Method of analysis for *Salmonella* and *Campylobacter*

Sample analysis for *Salmonella*

Caecal and rinse samples will be tested for *Salmonella* using the Australian Standard technique for *Salmonella* testing “AS 5013.10-2004: Food microbiology - Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp”.

Initial testing for *Salmonella*

Caecal and whole chicken rinse (collected at primary processor) samples will be analyzed for *Salmonella* qualitatively as follows:

1. Decant 100 ml of rinse into a suitable container and add equal volume of double strength buffered peptone water (BPW).
2. Mix and incubate at 37°C for 18 to 24 hours.
3. Transfer 0.1 ml of this culture to a tube containing 10 ml of Rappaport-Vassiliadis medium with soya (RVS broth) and 1 ml of the bacterial culture into a tube containing 10 ml of Muller-Kauffmann tetrathionate novobiocin broth (MKTTn broth).
4. Incubate the inoculated RVS broth at 41 ±1°C for 24 h ±3 h and the inoculated MKTTn broth at 37 ±1°C 24 ±3. Care should be taken that the maximum allowed incubation temperature of (42.5°C) is not exceeded.
5. After incubation for 24 ±3 h, using the culture obtained in the RVS broth, inoculate by means of a loop the surface of one large-size Petri dish containing the first selective plating-out medium, Xylose lysine deoxycholate agar (XLD agar) so that well-isolated colonies will be obtained. Proceed in the same way with the second plating-out medium using a sterile loop and Petri dish as described. The choice of the second selective medium is left to the specific laboratory undertaking the analysis. The standard does specify that manufacturer's instructions should be followed precisely.
6. After incubation for 24 ±3 h, using the culture obtained in the MKTTn broth, repeat the procedure described in step 5 with the two selective media.
7. Invert the dishes so that the bottom is uppermost, and place them in the incubator set at 37°C for the XLD agar and follow manufacturer's instructions for the second media.
8. After incubation for 24 ±3 h, examine the plates for the presence of typical colonies of *Salmonella* and atypical colonies that may be *Salmonella*.²⁶ Mark their position at the bottom of the dish. Incubate the second selective solid medium at the appropriate temperature and examine after the appropriate time to check for the presence of colonies which, from their characteristics, are considered to be presumptive *Salmonella*.

²⁶ The recognition of colonies of *Salmonella* is to a large extent a matter of experience, and their appearance may vary somewhat, not only from serovar, but also from batch to batch of the selective culture medium used. Typical colonies of *Salmonella* grown on XLD agar have a black centre and a lightly transparent zone of reddish colour due to the colour change of the indicator. However, *Salmonella* H₂S negative variants (eg *S. paratyphi* A) grown on XLD agar are pink with a darker pink centre. Lactose-positive *Salmonella* grown on XLD agar are yellow with or without blackening.

Quantitative *Salmonella* analysis- Most Probable Number technique

This procedure was developed by SARDI and introduces a substantial saving in media and generates results one day sooner than the traditional MPN method. The procedure is as follows:

1. Three sets of three tubes are inoculated as follows.
 - a. For whole chicken:
 - Set one – add 3 x 10.0mL of rinse fluid to each of three tubes containing 10mL double strength BPW.
 - Set two – add 3 x 1.0mL of rinse fluid to each of three tubes of single strength BPW.
 - Set three – add 3 x 0.1mL of rinse fluid to each of three tubes of single strength BPW.
 - b. For Caecal samples:
 - Set one – 3 x 1.0mL of a 1:10 dilution is added to three tubes 10mL single strength BPW
 - Set two – 3 x 1.0mL of a 1:100 dilution is added to three tubes of single strength BPW.
 - Set three – 3 x 1.0mL of a 1:1000 dilution is added to three tubes of single strength BPW.
2. Incubate the inoculated tubes at 37°C for 18 to 24 hours.
3. Spot inoculate 0.1mL of the incubated BPW onto 1/3 of a Modified Semisolid Rappaport-Vassiliadis (MSRV) medium and incubate upright at 42°C for up to and not exceeding 24 hours.
4. Examine the plates for motile bacteria indicated by a halo of growth emanating from the point of inoculation.
5. Where typical growth has occurred, subculture onto CLED agar from the edge of the halo.

Confirmation test

Salmonella will be confirmed serologically and biochemically as necessary. If shown to be reliable, commercially available identification kits for the biochemical examination of *Salmonella* may be used. The identification kits concerns the biochemical confirmation of colonies. These kits should be used following manufacturers instructions.

1. Selection of colonies for confirmation

- a. For confirmation, take from each Petri dish of each selective medium at least one colony considered to be typical or suspect and a further four colonies if the first is negative. It is recommended that at least five colonies be identified in the case of epidemiological studies. If on one dish there are fewer than five typical or suspect colonies, take for confirmation all the typical or suspect colonies.

- b. Streak the selected colonies onto the surface of pre-dried nutrient agar plates, in a manner which will allow well-isolated colonies to develop. Incubate the inoculated plates at $37\pm 1^{\circ}\text{C}$ for $24\text{ h}\pm 3\text{ h}$.
- c. Use pure cultures for biochemical and serological confirmation.

2. Biochemical confirmation

- a. Triple sugar/iron agar (TSI agar):
 - i. By means of an inoculating wire, streak the agar slant surface and stab the butt.
 - ii. Incubate at $37\pm 1^{\circ}\text{C}$ for $24\text{ h}\pm 3\text{ h}$.
 - iii. Interpret the changes in the medium as follows. Typical *Salmonella* cultures show alkaline (red) slants and acid (yellow) butts with gas formation (bubbles) and in about 90% of the cases formation of hydrogen sulphide (blackening of the agar). When lactose-positive *Salmonella* are isolated, the TSI slant is yellow. Thus, preliminary confirmation of *Salmonella* cultures shall not be based on the results of the TSI agar test alone.
- b. Urea agar (Christensen)
 - i. Streak the agar slant surface.
 - ii. Incubate at $37\pm 1^{\circ}\text{C}$ for $24\text{ h}\pm 3\text{ h}$ and examine at intervals.
 - iii. If the reaction is positive, splitting of the urea liberates ammonia, which changes the colour of phenol red to rose-pink and later to deep cerise. The reaction is often apparent after 2 to 4 h.
- c. L-Lysine decarboxylation medium
 - i. Inoculate just below the surface of the liquid medium.
 - ii. Incubate $37\pm 1^{\circ}\text{C}$ for $24\text{ h}\pm 3\text{ h}$.
 - iii. Turbidity and a purple colour after incubation indicates a positive reaction. A yellow colour indicates a negative reaction.
- d. Reagent for detection of β -galactosidase (or paper disks used in accordance to manufacturer's instructions).
 - i. Suspend a loopful of the suspected colony in a tube containing 0.25 ml of the saline solution.
 - ii. Add 1 drop of toluene and shake the tube. Put the tube in the water bath set at 37°C and leave for approximately 5 min. Add 0.25 ml of the reagent for detection of β -galactosidase and mix.
 - iii. Replace the tube in the water bath set 37°C and leave for $24\text{ h}\pm 3\text{ h}$, examine the tube at intervals.
 - iv. A yellow colour indicates a positive reaction. The reaction is often apparent after 20 min.
- e. Medium for Voges-Proskauer (VP) reaction
 - i. Suspend a loopful of the suspected colony in a sterile tube containing 3 ml of the VP medium.
 - ii. Incubate $37\pm 1^{\circ}\text{C}$ for $24\text{ h}\pm 3\text{ h}$.
 - iii. After incubation, add two drops of the creatine solution, three drops of the ethanolic solution of 1-naphthol and then two drops of the potassium hydroxide solution; shake after the addition of each reagent.
 - iv. The formation of a pink to bright red colour within 15 minutes indicates a positive reaction.

- f. Medium for indole reaction
 - i. Incubate a tube containing 5 ml of the tryptone/tryptophan medium with the suspended colony.
 - ii. Incubate at $37\pm 1^{\circ}\text{C}$ for 24 h ± 3 h.
 - iii. After incubation, add 1 ml of the Kovacs reagent.
 - iv. The formation of a red ring indicates a positive reaction. A yellow-brown ring indicates a negative reaction.

3. Serological confirmation and serotyping

In general, the detection of the presence of *Salmonella* O-, Vi- and H-antigens is tested by slide agglutination with the appropriate sera, from pure colonies and after auto-agglutinable strains have been eliminated. Use the antisera according to the producers' instructions if different from the description below.

- a. Elimination of auto-agglutinable strains
 - i. Place one drop of the saline solution onto a carefully cleaned glass slide.
 - ii. Disperse in the drop, by means of a loop, part of the colony to be tested, in order to obtain a homogenous and turbid suspension.
 - iii. Rock the slide gently for 30 s to 60 s.
 - iv. Observe the results against a dark background, preferably with the aid of a magnifying glass.
 - v. If the bacteria have clumped into more or less distinct units, the strain is considered auto-agglutinable and shall not be submitted to the following tests as the detection of the antigens is not feasible.
- b. Examination for O-antigens
 - i. Using one non-autoagglutinating pure colony, proceed as above, using one drop of the anti-O serum instead of the saline solution.
 - ii. If agglutination occurs, the reaction is considered positive.
- c. Examination for Vi-antigens
 - i. Proceed as above but using one drop of the anti-Vi serum instead of the saline solution.
 - ii. If agglutination occurs, the reaction is considered positive.
- d. Examination for H-antigens
 - i. Inoculate the semi-solid nutrient agar with a pure non-auto-agglutinable colony.
 - ii. Incubate the medium at $37\pm 1^{\circ}\text{C}$ for 24 h ± 3 h.
 - iii. Use this culture for examination for the H-antigens, proceeding as above but using one drop of the anti-H serum instead of the saline solution.
 - iv. If agglutination occurs, the reaction is considered positive.

Sample analysis for *Campylobacter*

Caecal and rinse samples will be tested for *Campylobacter* using the Australian Standard AS 5013.6 – 2004 “Food Microbiology Method 6: Examination for specific organisms – *Campylobacter*”. The details of this analysis are given below.

Initial testing for *Campylobacter*

Feed, cloacal swabs and rinse fluids will be tested for *Campylobacter* as follows:

1. Take 50 ml of the sample solution and add to 50 ml double strength Preston broth (without antibiotic supplement) and mix well.
2. Incubate the first dilutions and controls at $37\pm 1^\circ\text{C}$ for 2 h.
3. After the two hours incubation, add 0.4 ml of antibiotic supplement to each 100 ml of broth culture. The selective broths shall be incubated under microaerobic conditions at $42\pm 1^\circ\text{C}$ for 46 h.
4. Plating out on selective agar media. Inoculate each enrichment broth culture onto plates of preston agar and Skirrow agar as follows;
 - a. Using a 5 mm loop, streak the surface of each agar plate to obtain isolated colonies.
 - b. Incubate the plates at $42\pm 1^\circ\text{C}$ in a microaerobic atmosphere,
 - c. Examine the plates after 24 h and again after 48 h incubation. If suspect colonies²⁷ have formed, select three well-isolated colonies for the confirmation tests as described below.

Quantification analysis

The procedure to quantify *Campylobacter* numbers is as follows.

Procedure:

1. Inoculate 0.1 ml of unincubated first dilution onto plates of Preston agar and plates of Skirrow agar. Note that the two culture media are used in order to improve the recovery of *Campylobacter* according to the serotype present and the sample type being examined.
2. As controls, inoculate half plates of Preston agar and of Skirrow agar with each of the reference cultures (*Campylobacter jejuni* NCTC 11351 or ACM 3393 and *Campylobacter coli* NCTC 11366 or ACM 4983).
3. Incubate the plates at $42\pm 1^\circ\text{C}$ for 48 h and count the number of suspect colonies per plate.
4. For colony counts exceeding 100, select 10 random typical colonies on the presumptive plates; for colony counts below 100, select a number which approximates the square root of the count. Confirm the selected colonies by the methods described below.
5. Estimate the counts per unit mass or per unit surface are by the methods set out in AS 1766.3.1 and AS 5013.20 on both the Preston and Skirrow agars and record the higher of two counts and the agar medium on which it was obtained.

²⁷ *Campylobacter* colonies are smooth, flat, translucent and colourless to grey, with an irregular edge, pinpoint to 4 mm in diameter, often spreading along the streak line. Microscopic examination (dark field or phase contrast) of wet mounts from colonies of *Campylobacter* will show rapidly mobile, darting, curved S-shaped or spiral cells. Non-motile, non-culturable coccal forms may also be present.

Confirmation test

The procedure to confirm the presence of *Campylobacter* is as follows.

Procedure:

1. Culture selected colonies as follows:
 - a. Subculture each selected colony onto a separate blood agar plate
 - b. Incubate the inoculated plates in a microaerobic atmosphere at $42\pm 1^{\circ}\text{C}$ for 24 h or until growth is visible.
2. Oxidase test and Gram stain
 - a. Perform oxidase test (*Campylobacter* are oxidase positive)
 - b. Perform Gram stains on all oxidase-positive cultures, using dilute carbol fuchsin rather than sarasin as the counterstain.
3. Inoculate the following media as with the cultures that are Gram-negative and oxidase-positive as follows:
 - a. A tube of nutrient broth. Incubate under microaerobic conditions at $42\pm 1^{\circ}\text{C}$ for 24 h.
 - b. Two blood agar plates. Incubate one aerobically at $37\pm 1^{\circ}\text{C}$ and the other under microaerobic conditions at $25\pm 1^{\circ}\text{C}$. Incubate the plates for up to 5 days or until growth occurs if before than.
 - c. One blood agar plate with a heavy suspension in saline solution spread over the surface of the plate (lawn culture). Place a nalidixic acid disk and a cephalothin disc separately on the dried surface of the plate. Incubate at $42\pm 1^{\circ}\text{C}$ in a microaerobic atmosphere for up to 2 days.
 - d. The hippurate hydrolysis test procedure as follows:
 - i. Dispense 0.5 ml sterile sodium hippurate in a small screw-capped tube.
 - ii. Inoculate with a loopful of 24 hour culture from blood agar to provide a turbid suspension.
 - iii. Incubate tubes (including an uninoculated control) at $37\pm 1^{\circ}\text{C}$ for 2 h
 - iv. Add 0.2 ml of ninhydrin solution. Do not shake.
 - v. The development of a deep purple within 5 min is a positive result, development of a slight bluish colour is negative.
 - e. Inspect the incubated cultures and record the reactions observed in the following tests:
 - i. Motility – *Campylobacters* are motile in nutrient broth with the corkscrew or darting motion.
 - ii. Conditions of growth – *C. jejuni/C. coli* do not grow at 37°C aerobically or at 25°C under microaerobic conditions.
 - iii. Nalidixic acid sensitivity- *C. jejuni/C. coli* are sensitive to nalidixic acid.
 - iv. Cephalothin sensitivity – *C.jejuni/C.coli* are resistant to cephalothin. Sensitivity to nalidixic acid or cephalothin is indicated if the annular radius of the zone of inhibition is 6 mm or more.
 - v. Hippurate hydrolysis test – *C. jejuni* hydrolyses hiuppurate; *C. coli* does not.

Attachment 3: Form B: Sample Collection at Processing Facilities

FORM B: SAMPLE COLLECTION AT PROCESSING FACILITIES			
Baseline survey on the prevalence and levels of <i>Salmonella</i> and <i>Campylobacter</i> along the poultry meat supply chain			
<i>The following information is to be provided by all participating jurisdictions to allow analysis of data.</i>			
CONTACT DETAILS FOR PARTICIPATING JURISDICTION			
Name of sample collector:		Phone number:	
E-mail address:			
Organisation:			
INFORMATION ABOUT THE FACILITY BEING SAMPLED			
Facility being sampled:			
Has this facility previously been sampled? When?			
INFORMATION ABOUT THE SAMPLES			
Sample type (carcass rinse or carcass):			
Are there any circumstances whereby these samples could be compromised?			
Sample code	Sample temperature	Sample code	Sample temperature
1.		6.	
2.		7.	
3.		8.	
4.		9.	
5.		10.	
Further information about post-spin chill samples only:			
Type of wash and chill system			
Critical limits for controlling these systems			
pH of spin chiller at sample collection			
Chlorine concentration in the spin chiller at time of sampling			
Further information about caeca samples only:			
Crate time/use recorded for each sampling occasion			
Age of flock			
Number of previous pick-ups from the shed			
Time-off feed			

Attachment 4: *Salmonella* single and multiple serovar isolation profiles for positive samples

Table 4.1. *Salmonella* single and multiple serovar isolation profiles for positive samples

Serovar (combination)	Module 1: Rinse				Module 2: Caeca		Module 3 - Faeces	TOTAL
	NSW	QLD	SA	WA	SA	WA	WA	
Adelaide			10					10
Agona	2	2	16		2			22
Anatum						1	1	2
Braenderup							1	1
Give					1		3	4
Havana						2	10	12
Heidelberg							1	1
Infantis			32		13			45
Kiambu	18			9			9	36
Livingstone				1		1	16	18
Mbandaka	1	2	1	2		1	33	40
Montevideo	4		12					16
Muenchen	2						2	4
Muenster			24		3			27
Ohio			1				1	2
Reading	13	4						17
Senftenberg							1	1
Senftenberg z27					1			1

Sofia	67	56	41		33			197
Subsp 1 ser 16:1,v:-	7							7
Subsp 1 ser 4, 12:d	1							1
Subsp 1 ser rough:r:1,5			1					1
Tennessee					1		1	2
Typhimurium				35		9	5	49
Typhimurium 6			1					1
Typhimurium 6 var			2					2
Typhimurium 12a			1					1
Typhimurium 193					1			1
Typhimurium 29		1						1
Typhimurium 35			5		3			8
Typhimurium 135a			2		3			5
Virchow	1	1						2
Unknown						1		1
Double								
Adelaide & Montevideo			3					3
Adelaide & Muenster			1					1
Adelaide & Senftenberg			1					1
Infantis & Agona			1					1
Infantis & Montevideo			5					5
Infantis & Muenster			1					1
Infantis & Typhimurium 12a			1					1
Kiambu & Livingstone							3	3
Mbandaka & Havana							2	2
Mbandaka & Kiambu							7	7

Mbandaka & Livingstone							2	2
Mbandaka & Muenchen							1	1
Muenster & Montevideo			1					1
Muenster & Subsp 1 ser rough:r:1,5			3					3
Sofia & Agona					1			1
Sofia & Infantis					1			1
Sofia & Mbandaka	1							1
Sofia & Muenster			1					1
Sofia & Havana					1			1
Sofia & Reading	1							1
Sofia & Tennessee					1			1
Sofia & Virginia	1							1
Sofia subsp II & Give							2	2
Typhimurium & Mbandaka							3	3
Typhimurium 14+ & Kiambu							1	1
Typhimurium 6 & Typhimurium 6 va			9					9
Typhimurium 193 & Typhimurium 35					1			1
Triple								
Mbandaka & Kiambu & Tennessee							1	1
Mbandaka & Kiambu & Livingstone							1	1