Research Notes

Relative prevalence of *Salmonella* Sofia on broiler chickens pre- and postprocessing in Australia

G. E. Mellor,* L. L. Duffy,* G. A. Dykes,*1 and N. Fegan†2

*CSIRO Food and Nutritional Sciences, PO Box 3312, Tingalpa DC, Queensland, 4173, Australia; and †Food and Nutritional Sciences, 671 Sneydes Rd., Werribee, Victoria, 3030, Australia

ABSTRACT A survey was conducted to determine the relative prevalence of *Salmonella* serovars on whole chicken carcasses before and after processing in 3 Australian poultry abattoirs. Ninety and 180 whole chicken carcasses were tested for *Salmonella* serovars before and after processing, respectively. Each carcass was subjected to a buffered peptone water rinse according to Australian Standard methodologies and *Salmonella* prevalence was determined using Australian Standard methodologies. After isolation, *Salmonella* isolates were serotyped and results were analyzed to determine the relative percentage of each serovar at both processing points. *Salmonella* Sofia was shown to significantly increase its relative prevalence ($P \leq 0.05$) after processing and proved to be the dominant serovar accounting for 45/89 (51%) isolations before processing and 51/69 (74%) isolations after processing. The reasons for the increased relative prevalence of *Salmonella* Sofia are currently unknown and require further investigation but may involve factors related to prevalence and numbers on chickens and the ability of *Salmonella* Sofia to respond to environmental stressors and attach to surfaces.

**Key words:** *Salmonella* Sofia, prevalence, survival, poultry, processing

INTRODUCTION

*Salmonella enterica* ssp. II 1,4,12,27:b,[e,n,x] (*Salmonella* Sofia) is a geographically unique bacterial species that is consistently and almost solely isolated in Australia, most dominantly from poultry and poultry products (Sexton et al., 2007; Eglezos et al., 2008; Ponton et al., 2008). The association of this serovar with poultry is historically well established, from early epidemiological studies to more recent surveys, with many of these reporting high isolation rates from processed whole chicken and chicken products (Harrington et al., 1991; Sexton et al., 2007; Eglezos et al., 2008). In 2008, for example, the Australian *Salmonella* Reference Centre reported the serovars of 4,134 *Salmonella* isolates obtained from broiler chickens from across the country in that year. This report indicated that *Salmonella* Sofia was the most prevalent serovar, accounting for 45.9% of isolates, and *Salmonella* Typhimurium was the second most prevalent, representing 9.4% of isolates (IMVS, 2008). *Salmonella* Sofia is seldom isolated from cases of human disease and may be highly adapted to the poultry environment, as has been reported for other *Salmonella* serovars in different geographic regions (Chia et al., 2009b; Huehn et al., 2009). Established dominance of this serovar in poultry raises the possibility that it may reduce the burden of human disease associated with poultry-related *Salmonella* in Australia.

The majority of Australian poultry surveys to date have largely focused on endpoint or retail investigations and consequently there is a lack of published data available for the relative prevalence of *Salmonella* serovars preprocessing. Furthermore, it remains to be established if poultry processing in Australia affects the relative proportions of *Salmonella* serovars on poultry meat postprocessing. Despite this lack of data, the dominance of *Salmonella* Sofia on, and frequent isolation from, postprocessed chicken carcasses raises the possibility that this serovar has an enhanced ability to survive the hostile environment present within a poultry processing facility.

In Australia, poultry processing typically entails the following steps: killing, scalding, defeathering, evisceration, chilling, and packaging. Facilities may implement controls to help reduce bacterial load and minimize cross-contamination during these processes. The use of chilled chlorinated water during spin chilling is one such step that has been reported to decrease bacterial...
numbers on whole chicken carcasses, but the relative sensitivity of different serovars to such steps remains undetermined (Northcutt et al., 2003; Berrang et al., 2007, 2008).

This study was conducted to determine if the relative prevalence of *Salmonella* serovars on chicken carcasses changes between pre- and postprocessing, leading to the predominance of *Salmonella* Sofia isolated from Australian poultry.

**MATERIALS AND METHODS**

**Sampling**

Three Australian chicken abattoirs were used to establish the relative prevalence of *Salmonella* serovars on whole chicken carcasses pre- and postprocessing. Each abattoir was visited on 3 separate days between June and December 2007. On each visit, 10 carcasses with weights ranging from 2.14 to 2.96 kg were collected before scald, after spin chilling, and after packaging. Each bird was subjected to a whole carcass rinse following the method described in Australian Standard AS5013.20-2004, “Food microbiology—Preparation of test samples for microbiological examination—Poultry and poultry products” (Standards Australia, 2004a). Briefly, each bird was placed in a large sterile plastic bag with 500 mL of chilled buffered peptone water (Oxoid, Basingstoke, UK). The birds were shaken and massaged vigorously for 2 min, and the rinsate was collected in 250-mL sterile jars.

**Storage, Transport, and Processing**

After collection, samples were transferred chilled to the laboratory. On arrival, samples were stored at 2°C until processed. *Salmonella* were isolated using Australian Standard AS 5013.10-2004, “Food microbiology—Microbiology of food and animal feeding stuffs—Horizontal method for the detection of *Salmonella* spp.” (Standards Australia, 2004b). Briefly, 100 mL of rinsate was incubated at 37°C for 18 ± 2 h. A portion was added to both Rappaport-Vassiliadis with soy broth (bioMérieux, Marcy l’Etoile, France) and Muller-Kauffmann tetrahtionate-novobiocin broth (bioMérieux) and incubated at 41.5 or 37°C for 24 ± 3 h, respectively. Each enrichment was then streaked onto xylose lysine deoxycholate (bioMérieux) and brilliant green agar (bioMérieux) before incubation at 37°C for 24 ± 3 h. Up to 4 typical colonies were purified on nutrient agar (Oxoid) before confirmation using Microbact 24E kits as per the manufacturer’s instructions (Oxoid).

**Serotyping**

A maximum of 2 confirmed isolates were sent to the Institute of Medical and Veterinary Science (Adelaide, Australia) to be serotyped. Isolates were typed using slide agglutination according to the Kauffman and White *Salmonella* classification scheme. For each sample, the serotyping result from the first stored isolate was used in the analysis. *Salmonella* were grouped according to serovar and results were presented as percentage of *Salmonella* Sofia relative to other *Salmonella* serovars.

**Statistical Analysis**

Statistical analysis of combined plant results was performed using Fisher’s exact test (Minitab 15, Minitab Inc., Minneapolis, MN). This was done to determine whether there was a significant difference in *Salmonella* Sofia and *Salmonella* Typhimurium on carcasses pre- and postprocessing.

**RESULTS AND DISCUSSION**

The range and numbers of individual *Salmonella* serovars isolated from whole chicken carcasses in this study are presented in Table 1. The diversity of serovars present on carcasses decreased during processing, with 89 *Salmonella* encompassing 12 different serovars isolated before processing and 69 *Salmonella* encompassing 4 different serovars isolated after processing. *Salmonella* Sofia was the most frequently isolated serovar, accounting for 45/89 (51%) isolations before processing and 51/69 (74%) isolations after processing. *Salmonella* Typhimurium and *Salmonella* Chester were the second and third most frequently isolated serovars. *Salmonella* Typhimurium accounted for 22/89 (25%) and 16/69 (23%) before and after processing, respectively, whereas *Salmonella* Chester decreased in prevalence from 7/89 (7.9%) pre-processing to 0/89 (0%) post-processing. Most other serovars were isolated from low numbers of carcasses pre-processing and were undetectable post-processing. The relative percentage of *Salmonella* Sofia post-processing was significantly greater than pre-processing (*P* ≤ 0.05), whereas *Salmonella* Typhimurium was not significantly different (*P* ≥ 0.05). A closer analysis of changes in *Salmonella* Sofia prevalence pre- and post-processing is presented by plant run in Figure 1. Each run represents the sampling of a single flock before and after processing for each of the poultry plants. All runs in which *Salmonella* Sofia was detected before and after processing show an increase in the relative percentage of *Salmonella* Sofia on carcasses after production. Although demonstration of this feature is novel, the high isolation rate of *Salmonella* Sofia post-processing is consistent with Sexton et al. (2007), who demonstrated that 73% of sampled chicken carcasses exiting the screw chiller were positive for *Salmonella* Sofia. This prevalence is also consistent with the findings of Pointon et al. (2008), who demonstrated that *Salmonella* Sofia was the most prevalent serovar isolated from retail chicken samples in New South Wales and South Australia. In contrast to *Salmonella* Sofia, the relative prevalence of *Salmonella*
Typhimurium remained unchanged or decreased after processing (Figure 1).

Processing treatments established in plant A appeared to be more effective at reducing Salmonella than treatments established in plants B and C. Consequently, Salmonella were not isolated from postprocessed carcasses in the second run of plant A, resulting in a relative postprocessed Salmonella Sofia percentage of zero.

The relative prevalence of Salmonella serovars isolated from whole chicken carcasses during this survey changed notably when determined pre- as compared with postprocessing. In most cases, Salmonella Sofia increased its relative prevalence postprocessing, whereas the relative prevalence of the other serovars (e.g., Salmonella Typhimurium) remained unchanged or decreased. The authors suggest that a combination of factors including the status of Salmonella in flocks before processing, the status of Salmonella in the slaughter line before processing, environmental adaptability, attachment capability, presence or absence of flagella antigens, and poultry production systems may influence the survival of Salmonella throughout processing.

The microbiological status of preprocessed flocks may govern the relative prevalence and quantity of Salmonella serovars isolated from postprocessed carcasses. The authors suggest that a greater level of carcass cross-contamination may occur if the incidence and quantity of Salmonella Sofia in incoming broiler flocks is high. Unfortunately, there are few recent publications that review the epidemiology of Salmonella in Australian poultry flocks and preprocessed carcasses. Despite this,

Table 1. Total number and relative percentage of Salmonella serovars isolated from whole chicken carcasses

<table>
<thead>
<tr>
<th>Salmonella serovar</th>
<th>Number of Salmonella isolates before precool</th>
<th>Number of Salmonella isolates after chilling and packaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agona</td>
<td>2 (2.2)¹</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Anatum</td>
<td>1 (1.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Bovismorbificans</td>
<td>2 (2.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Chester</td>
<td>7 (7.9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Kiambu</td>
<td>2 (2.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mbandaka</td>
<td>1 (1.1)</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>Montevideo</td>
<td>1 (1.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Reading</td>
<td>1 (1.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Salmonella spp. I 4,12: d:-</td>
<td>1 (1.1)</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>Sofia</td>
<td>45 (50.6)</td>
<td>51 (74.0)</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>22 (24.7)</td>
<td>16 (23.2)</td>
</tr>
<tr>
<td>Virchow</td>
<td>4 (4.5)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

¹Numbers in parentheses indicate the relative percentage prevalence of individual serovars.

Figure 1. Salmonella Sofia and Salmonella Typhimurium isolated before and after processing expressed as a relative percentage of the total Salmonella isolated from each processing point, where n represents the total number of Salmonella isolated at each point (n also equals the number of Salmonella-positive carcasses). Samples were collected on 3 occasions (runs 1, 2, and 3) from 3 different plants (A, B, and C).
an early survey by Soerjadi-Liem and Cumming (1983) failed to isolate *Salmonella* Sofia from broiler flocks within a 30-km radius of a New South Wales processing plant, instead finding the predominant serovar to be *Salmonella* Typhimurium. Conversely, more recent anecdotal evidence suggests that *Salmonella* Sofia is the dominant serovar isolated from Australian poultry cecal content (M. Mackenzie, Inghams Enterprises Pty Ltd, Liverpool, New South Wales, Australia, personal communication). It is possible that *Salmonella* Sofia’s dominance in Australian poultry ceca occurred after the work conducted by Soerjadi-Liem and Cumming (1983) or that the survey mentioned was geographically limited because the dominance of *Salmonella* Sofia in processed chickens dates back to the late 1970s and early 1980s (Harrington et al., 1991).

In addition to carcass cross-contamination, it has also been suggested that contamination may result from previously contaminated slaughter lines. Rasschaert et al. (2007) have demonstrated that *Salmonella* serovars isolated from poultry processing plants before the commencement of processing can cross-contaminate *Salmonella*-free flocks during processing. A further study by Rasschaert et al. (2008) suggested that gastrointestinal-derived cross-contamination may not be as important as equipment or transport crate-derived cross-contamination.

Some *Salmonella* serovars have a range of defense mechanisms that allow them to survive environmental changes to factors such as pH, temperature, or nutrient availability. As mentioned by Humphrey (2004), “repeated exposure to a particular hostile environment will lead to the appearance of strains that are highly resistant to that environment.” The ability of *Salmonella* Senftenberg to withstand high temperature processing is an existing example of this (Manas et al., 2003). Similarly, *Salmonella* serotypes have been shown to persist for 5 d in a poultry processing line, which may suggest that efficient cleaning procedures are required to eradicate persistent *Salmonella* serovars (Olsen et al., 2003). It is possible that practices implemented within Australian poultry processing plants may provide conditions for which *Salmonella* Sofia is potentially better equipped to survive than the other serovars present on incoming poultry. *Salmonella* serovars may differ in their ability to attach to surfaces and evade stressors that may affect their survival throughout processing. It has already been demonstrated that *Salmonella* Sofia has a greater ability to attach to select inert materials than other serovars (Chia et al., 2009b). Although the experiments of Chia et al. (2009b) were conducted in a controlled environment, it is reasonable to infer that material type may also influence the ability of *Salmonella* Sofia to attach to surfaces used within poultry processing facilities.

Similar predominant serovars have been isolated from poultry internationally. Huehn et al. (2009) described *Salmonella enterica* type I serovar 4,12:d:- as a predominant serovar found in German poultry flocks. *Salmonella* strains harboring the 4,12:d:- antigen have also been commonly isolated from poultry in other European countries (Chadfield et al., 2001). Chia et al. (2009a) have suggested that *Salmonella* strains that harbor the 4,12:d:- antigen and lack phase 2 flagellar antigens are, as a result of these features, able to persist in Australian poultry.

Interestingly, and in contrast to Australian broiler chickens, *Salmonella* Sofia does not appear to colonize Australian egg-laying chickens (IMVS, 2008). There are no published data to clarify this; however, factors such as chicken breed, age, and feed type may be likely explanations. This observation suggests that poultry production systems may also contribute to *Salmonella* Sofia’s ability to survive in poultry.

The predominant *Salmonella* serovar isolated in the current study is not commonly associated with human disease and its high prevalence may result in reduced disease burden from *Salmonella* due to the consumption of poultry. It is clear that many factors influence the ability of *Salmonella* Sofia to colonize and survive in poultry throughout processing. This study demonstrates that *Salmonella* Sofia notably increases its relative prevalence on Australian chicken carcasses after processing. We suggest that this occurrence is unlikely to be due to a single factor but a combination of 2 or more of the following factors: prevalence and quantity on incoming chickens, ability to attach to surfaces found in poultry facilities, response to environmental stressors, presence and absence of flagella antigens, and poultry production systems.

**ACKNOWLEDGMENTS**

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