

ORIGINAL ARTICLE

Survival of *Salmonella* on refrigerated chicken carcasses and subsequent transfer to cutting board

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Abstract

Objective: To determine the effect of refrigeration time and temperature on *Salmonella* cell numbers on inoculated chicken carcasses and their transfer to a plastic cutting board.

Methods and Results: The survival of *Salmonella* on chicken skin and the transfer to a plastic cutting board when exposed to different refrigeration temperatures (2, 6 or 8°C) for 9 days were the two main issues on which this work focused. Two scenarios were carried out to ascertain these effects: carcasses treated with a decontaminating acetic acid solution and untreated carcasses. All of the contaminated carcasses remained contaminated after 9 days of refrigeration. However, on untreated samples, while *Salmonella* numbers increased almost 1.5 log at 8°C, the pathogen numbers decreased about 1 log at 2 and 6°C. On acid-treated samples, cell numbers slightly decreased at all of the temperatures studied. Temperature did not affect salmonellae transfer to the cutting board, but time did. Acid decontamination increased cell numbers transferred to the cutting board compared with untreated samples.

Conclusion: Proper refrigeration at low temperatures did not allow *Salmonella* numbers to rise, regardless of which carcasses had been, or had not been, acid treated. Despite the fact that the rate of transfer was not affected by temperature, the acid treatment detached *Salmonella* cells from the chicken skin and, therefore, the probability of greater cross-contamination should be studied further.

Significance and Impact of the Study: The results of this study may provide better information about the refrigeration conditions for fresh chicken storage and also determine if these, along with acetic acid decontamination of broiler chicken, would affect the pathogen transfer to a cutting board.

Introduction

Salmonella has been linked to many foodborne illness cases across the world, and it is considered to be one of the main agents causing human gastroenteritis in Latin America. In Argentina, the National Laboratory of Reference in Enterobacteria of the Department of Bacteriology – I.N.E.I. – A.N.L.I.S. – ‘Dr. Carlos G. Malbrán’, registered 19 foodborne illness outbreaks for the years 2004–2007 *Salmonella* being the causative agent: *Salmonella* Enteritidis (84.2% of the outbreaks), *Salmonella* Typhimurium

(10.5%) and *Salmonella* Heidelberg (5.3%). The number of people affected in these outbreaks is unknown (Caffer, M.I. and Terraño, R. (2007) Personal Communication from Servicio Enterobacterias – Laboratorio Nacional de Referencia de Enterobacterias – Departamento Bacteriología – I.N.E.I. – A.N.L.I.S. ‘Dr. Carlos G. Malbrán’, Buenos Aires, Argentina). The foods most likely implicated were poultry and egg products.

The Global *Salmonella* Surveillance Progress Report, 2005 (Binsztein *et al.* 2006) reported that *Salm.* Enteritidis was the dominant and most frequent serovar

worldwide, followed by *Salm.* Typhimurium and *Salm.* Newport.

Salmonella in broiler meat is considered to pose a serious risk to public health (Evers 2004). The percentage of carcasses positive for the pathogen varies substantially from one country to another, ranging from 5% to 21%, depending on, among other things, the sampling point (Anon. 2008; Kegode *et al.* 2008). Some EU countries, such as Denmark, Finland, Ireland and Norway have for several years had programmes put into practice aimed at controlling *Salmonella* in broilers, with some of them reporting very low levels of the pathogen over the last 5 years (Anon. 2008).

From the food safety perspective, it is necessary to consider that a microbiological hazard not only depends on the prevalence but also on the concentration of the pathogen found in a particular food. There is often an unusual factor, such as a raw product with a high-load, high-virulence pathogen or consumers who leave food exposed for too long in an environment that is too warm. The risk increases when a pathogen appears whose presence signals unacceptable likelihood of cross-contamination. Bloomfield and Scott (1997) stated that the cross-contamination risks associated with different sites and surfaces depend not only on the frequency of occurrence of potentially harmful organisms but also on the probability of transfer of contamination from that site.

Raw meat that is contaminated with foodborne pathogens is commonly handled in the kitchen. The load of this contamination is frequently low; therefore, the number of cells transferred to surfaces in contact with food is also low. Although it is believed that it would be necessary for this probable low transfer amount to be worsened by temperature abuse, high virulence, susceptible consumer or a long storage period in order to be considered a significant health risk, there are few data available which allow us to quantify the rate of transfer of pathogens from food to cutting boards or other food contact surfaces (de Boer and Hahné 1990).

The objective of this study was to obtain laboratory data from inoculated chicken carcasses with which the effect of time and temperature of refrigeration on *Salmonella* numbers and rate of transfer could be quantified. Two scenarios were analysed during the study: carcasses treated with a decontaminating acetic acid solution and untreated carcasses.

The results of this study may provide better information about the refrigeration conditions for fresh chicken storage, and also determine to what extent these, along with decontamination of broiler chicken as a useful

intervention, would affect the pathogen transfer to cutting boards.

Materials and methods

Samples

Chilled chicken carcasses were obtained from a local government-inspected poultry processing plant and maintained in a refrigerator while they were transported to the laboratory. Samples were negative to naturally occurring *Salmonella*.

Preparation of inocula

Pure cultures of *Salmonella enterica* subspecies *enterica* serotype Hadar isolated and identified in a previous study on chicken carcasses were cultured at 37°C for 24 h in tryptic soy broth (Difco, Buenos Aires, Argentina). After that, they were centrifuged at 1533 g for 10 min, and the cell pellet was then suspended in 0.1 mol l⁻¹ potassium phosphate buffer (pH 7.0) to give a viable cell population of around 10⁷ CFU ml⁻¹, calculated by plate count on XLD (Merck, Buenos Aires, Argentina). Finally, the plates were incubated at 35°C for 24 h.

Samples preparation procedure

Two 25 cm² areas of the skin of chicken breast were inoculated each with 0.1 ml aliquots of a 24 h culture of *Salm.* Hadar and spread over the limited areas. The areas were marked off in order to assign one area to the survival assay and the adjacent area to the transfer assay.

The inoculated samples were dried in a laminar, sterile air-flow chamber for 20 min in order to allow enough time for the micro-organism to attach. After that, samples which received no decontamination treatment were immediately and individually placed in plastic bubble-shaped trays and stored in the refrigerator at their corresponding assay temperatures (2, 6 or 8°C) and the same amount of samples were submitted for decontamination treatment.

Acid-treated samples

The inoculated samples submitted for decontamination treatment were sprayed for 15 s with a 2% acetic acid solution and drained for 1 min. The temperature of the spraying solution was 30°C. Then, they were placed in individually plastic bubble-shaped trays and stored at their respective assay temperatures (2, 6 or 8°C).

Microbiological analysis

Salm. Hadar counts were taken on the day of inoculation and after 1, 3, 5, 7 and 9 days storage for survival and transfer assays.

Survival assay

Every sampling day, one of the inoculated chicken skin areas was cut with a scalpel and removed with a sterile forceps and placed in 90 ml of buffered 0.1% peptone water and shaken for 2 min. Aliquots of 0.1 ml of appropriate suspension dilutions were then placed on XLD and incubated for 24 h at 35°C according to the media recommended by the Bacteriological Analytical Manual (BAM-FDA) (Andrews and Hammack 1998).

Transfer assay

The other 25 cm² inoculated area was cut with its underlying flesh attached. It was placed with the skin down and smeared over a 25 cm² plastic cutting board (Crom, Argentina). After the cutting board was contaminated, the area was sampled with a dacron-tipped swab and then rinsed in peptone water. The swab was wetted and rubbed across the surface in one direction, rinsed in 5 ml peptone water and rubbed in the opposite direction and rinsed for a second time (Snyder 1997). Aliquots of 0.1 ml of appropriate dilutions were then placed on XLD and incubated for 24 h at 35°C according to the media recommended by the BAM method (Andrews and Hammack 1998).

Sensory observations

Sensory observations by an untrained panel were used to evaluate the odours and characteristics of the chicken skin in the over-wrapped tray samples throughout the entire storage periods.

Statistical analysis

All experiments were performed twice at each temperature assayed and proposed scenarios. Two samples were analysed on each sampling day.

Counts were expressed as log colony forming units (CFU) per 25 cm² (skin). Data were subjected to analysis of variance (ANOVA), and Duncan's multiple range test was used to determine differences between means.

Results

Table 1 shows *Salm.* Hadar mean counts on chicken skin and counts transferred to the cutting board at the day of inoculation.

Table 1 *Salmonella* mean counts on chicken skin and transferred to cutting board at the day of inoculation

Samples	<i>Salmonella</i> mean counts†
Acetic acid treated chicken skin carcasses	4.4 ± 0.6 ^b
Transferred to cutting board from acetic acid treated chicken skin carcasses	3.2 ± 0.5 ^c
Untreated chicken skin carcasses	5.4 ± 0.5 ^a
Transferred to cutting board from untreated chicken skin carcasses	4.0 ± 0.4 ^b

†Log₁₀ CFU skin⁻¹ ± SD. Means with the same letter are not significantly different ($P > 0.05$).

On day 7 and 9 of storage, higher salmonellae counts were found on untreated carcasses stored at 8°C ($P \leq 0.05$) than on samples at 2 and 6°C.

By day 9 of storage at 8°C, numbers of *Salm.* Hadar inoculated on chicken skin multiplied slowly on untreated samples and reached 6.6 log CFU per skin, but a constant and slight decrease in numbers took place on acid-treated carcasses, and these attained 3.7 log CFU per skin. In addition, for chicken carcasses stored at 2 and 6°C, survival was not found to be significantly higher ($P > 0.05$) on untreated carcasses (about 4 log CFU per skin) than on decontaminated carcasses (about 3.5 log CFU per skin).

It can be said that there was no significant effect ($P > 0.05$) on the pathogen numbers due to temperature shifts on chicken samples decontaminated with acetic acid. However, every temperature studied slightly decreased salmonellae numbers during the refrigeration periods.

By day 9 of storage, both treated and untreated samples developed off-odours at all temperatures assayed. Nevertheless, on untreated samples, while an incipient spoiled condition became evident at 2°C from day 7 on, off-odours were apparent at 6 and 8°C from day 5. On acid-treated chicken carcasses, slight off-odours were evident at the end of the assays at 2 and 6°C. At 8°C, considerably perceptible off-odours came up from day 7 and stronger by day 9.

Salmonellae were more easily transferred to surfaces from acid-decontaminated carcasses than from untreated samples. However, transference to a surface was more marked on carcasses at the day of inoculation than on subsequent days of storage. As it can be seen in Figs 1 and 2, while time did affect salmonellae transfer to a cutting board in both scenarios assayed, temperature affected only the untreated samples.

Discussion

This research studied the refrigeration conditions affecting the survival of *Salm.* Hadar inoculated on chicken skin

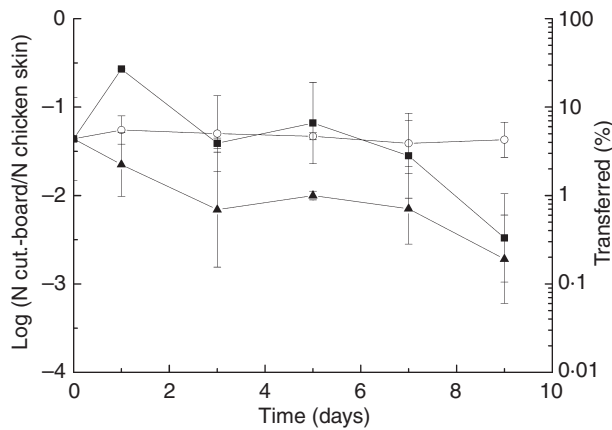


Figure 1 Transfer of *Salmonella* from untreated carcasses and stored at 2, 6 or 8°C, to cutting board.

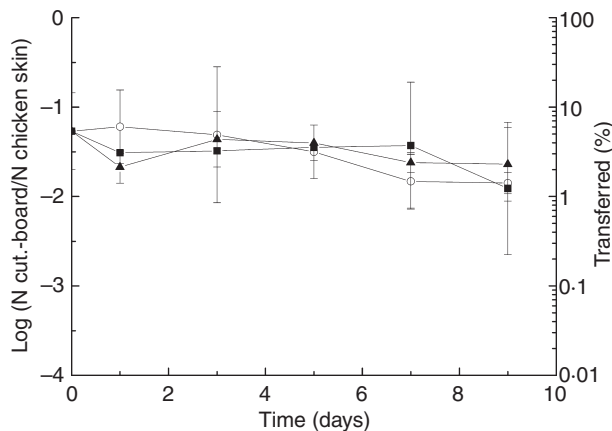


Figure 2 Transfer of *Salmonella* from carcasses decontaminated with 2% acetic acid and stored at 2, 6 or 8°C, to cutting board.

and the potential of transfer to a plastic cutting board after two possible processing scenarios: carcasses treated with acetic acid solution or untreated carcasses. The scenarios were designed to establish if the decontamination with acetic acid contributed to the reduction of *Salmonella* at each temperature selected and the potential transfer to contact surfaces. The refrigeration temperatures were chosen to reproduce a government-recommended temperature guidance for meat (2°C), a common temperature in domestic or retail service equipment (6°C) and a temperature abuse situation (8°C).

Although the addition of a decontamination treatment ensured no growth of *Salm.* Hadar on chicken skin that had been exposed to chill temperatures in the refrigerator, regardless of temperature assayed, considerable numbers of the pathogen survived and remained viable for up to 9 days. Further, only a slight decrease in cell numbers was found at every temperature assayed on decontaminated

carcasses. Jackson *et al.* (2007) reported that food pathogens can survive on refrigerator surfaces and could therefore pose a cross-contamination risk.

On the other hand, Mattick *et al.* (2003) reported that *Salm.* can survive air drying for at least 24 h, and consequently, when many bacteria are released from perishable food and they are viable for a long time on the cutting board, it may mean a higher cross-contamination potential. This agrees with Dawson *et al.* (2007) who stated the ability of bacteria to survive and cross-contaminate other foods even after long periods of time on dry surfaces, thus reinforcing the importance of sanitizing food contact surfaces to minimize the risk of foodborne illness.

In this study, higher *Salm.* Hadar numbers were detected on a cutting board surface from decontaminated samples than from untreated ones. It appears that the bacteria detach from chicken skin more easily when an acid treatment has been carried out and therefore, it is transferred more frequently than from untreated samples at 6 and 8°C ($P \leq 0.05$).

Nissen *et al.* (2001) found that *Salm.* Enteritidis inoculated on chicken breast muscle with skin attached grew rapidly and reached $\log 7$ CFU cm^{-2} after 4 days of storage at 10°C. These authors also reported that growth was not significantly different on decontaminated and untreated chicken, except after 3 days, when a significantly higher growth was detected on the untreated samples. This agrees with the results obtained here, but only for the samples stored at 8°C. However, lower cell numbers were attained in our study than those obtained by Nissen *et al.* (2001) by day 4, and they were still low even at day 9. The difference in the cell counts found in these studies may be due to the different temperatures assayed in both investigations.

Similar to Dickson (1990), who stated that the moisture content of meat plays an important role in the attachment of bacteria, in our study, the transference to surface was more marked when carcasses were still fresh and the moisture of the skin was high. During storage, the chicken skin got dry and adhesive, regardless of whether the carcasses were treated or untreated, and the transfer frequency decreased as a function of time. These results agree with those obtained by de Boer and Hahné (1990) and Kusumaningrum *et al.* (2003).

As stated above, when chicken carcasses were held at low temperatures or even in the worst of conditions/scenarios (8°C), decontaminated samples showed a slight decrease in salmonellae numbers during the whole period of storage. Moreover, mild off-odours developed at the end of the study at each temperature assayed. These results do not agree with those of Nissen *et al.* (2001), who stated that there is a risk that decontamination will allow increased multiplication of the pathogen because of the extended shelf-life. This was not the situation in

decontaminated carcasses up to 9 days of storage, according to the results of our investigation.

Conclusions

Decontamination of chicken carcasses may be a useful intervention when reduction of bacteria is the main concern, but the acid treatment detached salmonellae cells from the chicken skin, allowing for contamination of the cutting board. Therefore, the probability of greater cross-contamination should be studied further.

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