PERSISTENCE OF *SALMONELLA* SEROTYPES ON CHICKEN SKIN AFTER EXPOSURE TO KOSHER SALT AND RINSING*

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ABSTRACT

A series of experiments was undertaken to determine whether kosher salt reduces persistence of Salmonella *serotypes that might cross-contaminate chicken skin on the conveyor belt between the soaking and salting stations in a kosher processing line. The line was simulated in the laboratory because* Salmonella *could not be inoculated onto chickens in a commercial plant. Prevalence of* Salmonella Typhimurium *(0.5 $\log_{10}$ cfu) was reduced (P < 0.0001) from 93 to 21% by kosher salt followed by rinsing as compared with 48% for rinsing alone; results were similar for* Salmonella Kentucky *and for 12 and 24C.* Salmonella Hadar *was less persistent than the other serotypes. The beneficial effect of kosher salt on reducing persistence of* Salmonella *was not observed when initial pathogen levels were greater than 2.5 $\log_{10}$ cfu and when kosher salt was applied without rinsing. These results suggest that the application of kosher salt followed by rinsing is an important pathogen reduction step in the kosher processing of chickens.*

PRACTICAL APPLICATIONS

Jewish dietary laws have been in existence for thousands of years but have received little attention from the scientific community for their potential beneficial effects for improving the quality and safety of poultry products.

* Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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Practices employed during the kosher processing of poultry may be amendable to non-kosher processing of poultry. Results of this study suggest that application of dry kosher salt might reduce attachment of *Salmonella* during processing and facilitate their removal by carcass washing. Further studies are needed to validate these results in a commercial plant and to evaluate other types of salt for their effects on *Salmonella* persistence and the potential application of the process to non-kosher poultry.

INTRODUCTION

Shriah is a step in kosher processing in which poultry are soaked in cold water to remove coagulated blood (Zuckerman and Abraham 2002). Water temperatures of 8–12C and soaking times of 30–60 min are common (Zuckerman and Abraham 2002). After Shriah, poultry are conveyed by belt in large commercial plants to the station where dry kosher salt is applied by hand to both the inside and outside of the bird. Carcasses are then piled onto a slow-moving conveyor belt that transports them to the rinsing stations, a trip that lasts about 1 h during which the salt extracts additional blood (Anon 1980). A poultry washer is used to rinse salt from the surfaces before two sequential rinses in communal water baths (8–12C) to complete salt removal (Katz 1981). Cross-contamination with *Salmonella* during these steps of kosher processing, collectively called kashering, is a concern to processors, the United States Department of Agriculture inspectors and consumers.

Bacterial contamination of skin or meat surfaces during processing occurs in two stages (Firstenberg-Eden 1981). In the first stage, bacteria in the water layer bind to sites on the meat or skin surfaces and are loosely attached. In the second stage, bacteria become firmly attached by forming a biofilm through production of an extracellular matrix. Rate and firmness of attachment are time dependent (Firstenberg-Eden *et al.* 1978) and are affected by bacterial strain (Notermans and Kampelmacher 1974), type of meat surface (Firstenberg-Eden *et al.* 1978), pH (Notermans and Kampelmacher 1974), temperature (Notermans and Kampelmacher 1974), physiological state of bacteria (Firstenberg-Eden 1981) and other factors (Firstenberg-Eden 1981; Notermans and Kampelmacher 1974).

Chicken skin is one of the best surfaces for attachment of bacteria (Firstenberg-Eden *et al.* 1978). Implications for poultry processing are that firmly attached and entrapped bacteria are much more difficult to remove by chemical disinfections and washing (McMeekin *et al.* 1984; Thomas *et al.* 1987), but they are less likely to cause cross-contamination of equipment and other poultry (Firstenberg-Eden *et al.* 1978). On the other hand, attached and entrapped bacteria are less readily detected by carcass sampling methods

involving swabbing and rinsing (Patterson 1972; Notermans and Kampelmacher 1975), thus causing an overestimate of food safety.

Initial binding of bacteria to meat surfaces involves ionic interactions (Firstenberg-Eden 1981) and, as such, saline rinses can reduce firm attachment of *Salmonella* to chicken skin (McMeekin *et al.* 1984). An interesting, but unexamined, question is whether kosher salt alters persistence of *Salmonella* on chicken skin. It is possible that kosher salt could reduce bacterial attachment by interfering with the initial stage of attachment and result in enhanced removal of cross-contaminating *Salmonella* during subsequent rinsing. The objective of this research was to determine whether kosher salt reduces persistence of *Salmonella* serotypes that might cross-contaminate chicken skin on the conveyor belt between the soaking (Shriah) and salting stations in a kosher processing line, which in this study was simulated in the laboratory because *Salmonella* could not be inoculated onto chickens in a commercial plant.

MATERIALS AND METHODS

Salmonella

Chicken skin portions were inoculated with multiple antibiotic resistant (MAR) *Salmonella enterica* serotypes; Typhimurium definitive phage type 104 (DT104; ATCC 700408, American Type Culture Collection, Manassas, VA), Kentucky (poultry isolate) or Hadar (poultry isolate). Stock cultures of the MAR *Salmonella* were maintained at –70C in brain–heart infusion (BHI; Difco Laboratories, Detroit, MI) broth that contained 15% (v/v) glycerol (Sigma Chemical Co., St. Louis, MO). Multiple antibiotic-resistant *Salmonella* were used because it is possible to follow low levels of them on chicken skin with natural microflora by using selective plating media with multiple antibiotics (Oscar 2006).

Chicken Skin

Chicken thighs were purchased weekly from local retail outlets. The skin was removed, spread on a cutting board and then frozen at –20C for 15 min. A number 10-cork borer was used to cut circular pieces of frozen skin (2.14 cm^2) for challenge studies. Freezing was carried out to facilitate cutting of the skin.

Salmonella Cultures

Thawed stock culture (5 µL) was added to the BHI broth (5 mL) in an Erlenmeyer flask (25 mL) that was sealed with a foam plug. Cultures were

then incubated at 30C for 23 h and 150 rpm to obtain stationary phase cells. Before inoculation of skin portions, cultures were serially diluted to 10^{-7} in buffered peptone water (BPW; Difco).

Process Simulation

Skins portions were inoculated with 2 µL of a 10^{-7}, 10^{-6}, 10^{-5} or 10^{-4} dilution of the appropriate *Salmonella* culture. Before application of kosher salt, skin portions were incubated at 12 or 24C for 15 min to simulate cross-contamination of chickens with *Salmonella* on the conveyor belt between soaking and dry salting in a kosher processing plant. Kosher salt was applied ($^1/_8$ tsp or 1.19 ± 0.05 g/portion or 0.55 ± 0.02 g/cm^2; mean ± SEM) or not, and then, skin portions were held at 12 or 24C for 60 min to simulate transfer from the salting station to the rinsing stations in a kosher processing plant. Before determination of *Salmonella* prevalence, skin portions were transferred or not to a specimen cup (250 mL) that contained 100 mL of spring water (Snow Valley, Inc., Upper Marlboro, MD) and held at 12 or 24C. Skin portions were rinsed in this communal bath for 15 min at 150 rpm to simulate rinsing in a kosher processing plant.

Kosher salt is coarser than "regular" salt and does not contain additives. The larger grain size, which is variable, is on average twice the size of "regular" salt. Because kosher salt has larger grain sizes, it is less soluble in water and better able to extract blood from the carcass than "regular" salt.

Prevalence Assay

After treatment, individual skin portions were transferred to stomacher bags that contained BPW (40 mL). Samples were pulsified (Pulsifier model PUL 100, Microbiology International, Frederick, MD) for 10 s and incubated at 38C for 24 h. After incubation in BPW, skin sample enrichment (2 µL) was spot inoculated onto XLH-CATS for *S. Typhimurium*, XLH-NATS for *S. Kentucky* and XLH-TUGS for *S. Hadar*. All three were xylose lysine (XL) agar media that contained 25 mM N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] or HEPES (H) and four of the following seven antibiotics (25 µg/mL each): chloramphenicol (C), ampicillin (A), tetracycline (T), streptomycin (S), novobiocin (N), sulfasoxazole (U) or gentamicin (G). All media supplements were from Sigma. It should be noted that since the *Salmonella* were pre-enriched in BPW for 24 h before spot inoculation onto selective media with multiple antibiotics, the assay allowed for recovery of injured cells, and thus, decreases in prevalence were not over-estimates of food safety.

Statistical Analysis

Prevalence of *Salmonella* among treatments was compared using a chi-square test (version 4.0 of Prism, GraphPad Software, Inc., San Diego, CA).

RESULTS

An initial set of experiments was conducted to determine the effect of kosher salt and rinsing on persistence of a low initial dose of *S. Typhimurium* on chicken skin held at 24C (Table 1). Results of Experiment I indicated that *Salmonella* prevalence was not altered ($P > 0.05$) by exposure to kosher salt alone, whereas results of Experiment II indicated that *Salmonella* prevalence was reduced ($P < 0.0001$) by exposure to kosher salt followed by rinsing. In Experiment III, *Salmonella* prevalence was reduced ($P < 0.0001$) to a greater extent by kosher salt followed by rinsing than by rinsing alone, which confirmed the combined results of Experiments I and II.

To demonstrate that the results of Experiment III were not unique to serotype Typhimurium, the experiment was repeated with serotype Kentucky (Table 2). Similar to the results of Experiment III, results of Experiment IV with a low initial density of *S. Kentucky* indicated that kosher salt followed by rinsing resulted in a greater reduction ($P < 0.0001$) of *Salmonella* prevalence than rinsing alone.

In the next set of experiments, the effect of initial dose on the persistence of *S. Typhimurium* on chicken skin that was exposed to kosher salt and then rinsed was examined (Table 3). Results of Experiments V and VI indicated that kosher salt followed by rinsing reduced ($P < 0.0001$) the prevalence of *S. Typhimurium* at the two lowest initial doses but not at the two highest initial doses and that the results were similar at 12 and 24C.

TABLE 1.
EFFECT OF KOSHER SALT AND RINSING ON PERSISTENCE OF *SALMONELLA TYPHIMURIUM* ON CHICKEN SKIN AT 24C

Experiment	Dose ($\log_{10}$ cfu)	Treatment	Prevalence	Positive samples	Total Samples	Chi-square
I	0.5	Control	93	56	60	$P = 0.12$
		Salt	84	49	58	
II	0.4	Control	92	37	40	$P < 0.0001$
		Salt + Rinse	12	5	40	
III	0.4	Control	93	93	100	$P < 0.0001$
		Rinse	48	48	100	
		Salt + Rinse	21	21	100	

TABLE 2.
EFFECT OF KOSHER SALT AND RINSING ON PERSISTENCE OF *SALMONELLA KENTUCKY* ON CHICKEN SKIN AT 24C

Experiment	Dose (log_{10} cfu)	Treatment	Prevalence (%)	Positive samples	Total samples	Chi-square
IV	0.4	Control	92	46	50	$P < 0.0001$
		Rinse	44	22	50	
		Salt + Rinse	10	5	50	

TABLE 3.
EFFECT OF DOSE AND TEMPERATURE ON PERSISTENCE OF *SALMONELLA TYPHIMURIUM* ON CHICKEN SKIN AFTER KOSHER SALT AND RINSING

Experiment	Temperature	Dose (log_{10} cfu)	Prevalence (%)	Positive samples	Total samples	Chi-square
V	24C	0.5	16	8	50	$P < 0.0001$
		1.5	60	30	50	
		2.5	96	48	50	
		3.5	100	50	50	
VI	12C	0.7	8	5	60	$P < 0.0001$
		1.7	55	33	60	
		2.7	100	60	60	
		3.7	100	60	60	

In the final set of experiments, the effects of kosher salt and rinsing on the persistence of serotypes Typhimurium and Hadar on chicken skin held at 12C were investigated (Table 4). Similar to the results of previous experiments, kosher salt alone did not alter the prevalence of *Salmonella*, and kosher salt followed by rinsing resulted in a greater reduction of *Salmonella* prevalence than rinsing alone. In contrast to the experiments comparing serotypes Typhimurium and Kentucky, these experiments indicated that serotype Hadar was less persistent than serotype Typhimurium when kosher salting was followed by rinsing.

DISCUSSION

The use of 12C as an incubation/rinsing temperature was based on water temperature data obtained from a cooperating kosher poultry processing plant. These data indicate that cold water used in kosher processing has a temperature range of 50–55F (10–12.8C). The incubation temperature of 24C used in

TABLE 4.
EFFECT OF SEROTYPE ON PERSISTENCE OF *SALMONELLA* ON CHICKEN SKIN AFTER KOSHER SALT AND RINSING AT 12C

Experiment	Serotype (dose)	Treatment	Prevalence (%)	Positive samples	Total samples	Chi-square
VII	Typhimurium	Control	97	35	36	$P < 0.0001$
	(0.5 $\log_{10}$ cfu)	Salt	89	32	36	
		Rinse	64	23	36	
		Salt + Rinse	28	10	36	
VIII	Hadar	Control	94	34	36	$P < 0.0001$
	(0.6 $\log_{10}$ cfu)	Salt	100	36	36	
		Rinse	64	23	36	
		Salt + Rinse	6	2	36	

some of our experiments was also based on data obtained from a kosher processing plant. These data indicate that between soaking, dry salting and rinsing, core product temperature ranges from 60 to 86F (15.6–30C) with a most likely value of 75F (23.9C). Thus, these experiments simulated product temperatures that might be encountered from the core to the surface during the kashering (soaking, dry salting and rinsing) segment of kosher processing of poultry. Effects of kosher salt and rinsing on persistence of *Salmonella* serotypes were similar at 12 and 24C in this study.

The level of kosher salt used in this study was, on average, 0.55 g/cm^2. In comparison, the average amount of kosher salt applied to chickens in a commercial plant was determined to be 0.12 g/cm^2 ($n = 8$; range 0.08–0.18 g/cm^2). Although the amount of salt used in the current study was, on average, 3.6-fold higher than that used in a commercial plant, both application rates resulted in complete coverage of the skin with kosher salt. Thus, the higher amount of kosher salt used in this study likely did not affect the results as most of it was not in direct contact with the skin surface. Moreover, when kosher salt was applied alone without rinsing, there was no observed effect on persistence of *Salmonella*.

The scientific literature contains a limited number of reports on the kosher processing of poultry. Levinger and Soroker (1974) investigated the effect of a chemical agent on removal of feathers from chickens immersed in cold water during kosher processing. Angel and Weinberg (1986) and Angel *et al.* (1989) studied the uptake of salt by chicken carcasses during kosher processing. They observed significant variations in salting practices and outcomes among kosher plants in Israel. Zuckerman and Abraham (2002) demonstrated efficacy of antimicrobial treatments against *Listeria monocytogenes* and other bacteria that contaminate chickens during kosher processing. These

few studies demonstrate the limited knowledge that exists about how kosher processing affects quality and safety of poultry.

Results of this study suggest that application of kosher salt might reduce attachment of *Salmonella* attempting to cross-contaminate poultry during processing, resulting in enhanced removal during carcass washing and rinsing. In fact, salt has been shown to prevent attachment of *Salmonella* to chicken meat surfaces (Thomas and McMeekin 1981). Proposed mechanisms for this effect are that salt extracts bacteria-glycosaminoglycan complexes from the meat surface and that high salt reduces water retention and the associated physical retention of bacteria by exposed connective tissues on the chicken meat surface (Thomas and McMeekin 1991). Thus, these results suggest that proper salting (i.e., complete coverage of the chicken carcass surface with kosher salt) of chickens and other poultry during kosher processing is an important process control step that can minimize contamination of the final product with *Salmonella* and perhaps other pathogens that might cross-contaminate the bird during commercial processing operations.

Studies that investigate the efficacy of interventions for reducing levels of pathogenic bacteria often involve determinations of log cycle reductions of target organisms artificially inoculated at high initial doses (10^6–10^8 cfu) onto the food surface. In the current study, rather than assess $\log_{10}$ cycle changes in *Salmonella* numbers, we assessed changes in prevalence. This alternative approach to assessing food safety was used because the current practice in the poultry industry is to measure prevalence rather than numbers of *Salmonella*. In addition, by looking at prevalence rather than $\log_{10}$ cycle reductions, we were able to much more easily assess the effects of our simulated kosher process on persistence of low and ecological levels of *Salmonella* (i.e., <10 cfu). An advantage of this approach is that the natural ecology of the chicken was preserved in the challenge studies, resulting in findings that are potentially more relevant to commercial practice. Moreover, inoculation with high levels of the test organism might create a best-case scenario for the process being evaluated, resulting in an overestimation of process control and food safety. For example, if under native conditions there are 1 pathogen and 1,000 other bacteria per cm^2 of food surface and one lethal molecule of the test intervention (e.g., chemical disinfectant) interacts with the subject food surface, then there is a 1 in 1,001 chance that the target process molecule will hit the pathogen. If, on the other hand, 999 pathogens are artificially inoculated onto the subject food surface, the odds of the target process molecule hitting a pathogen cell is now improved artificially to 1 in 2.

In the current study, a wide range of *Salmonella* doses from 0.4 (2.5 cfu) to 3.7 (5,011 cfu) $\log_{10}$ cfu per 2.14 cm^2 of chicken skin were investigated. These levels correspond to those reported for non-kosher chickens. D'Aoust *et al.* (1982) reported that chickens contain on average 1.8 cells of *Salmonella*

per 100 g of skin. Dougherty (1974) found that *Salmonella* levels on chicken skin range from 43 to greater than 1,100 cells per cm^2. Surkiewicz *et al.* (1969) found that levels of *Salmonella* contamination on chicken were typically 1–30 cells per bird, with some birds having more than 300 cells of *Salmonella*. Results of the present study indicate that kosher salt followed by rinsing did not reduce prevalence when *Salmonella* levels 2.5 $\log_{10}$ cfu (316 cfu) or higher were inoculated onto chicken skin portions (2.14 cm^2). However, significant reductions in prevalence of *Salmonella* contamination were observed when the inoculated level of *Salmonella* contamination was less than 1.7 $\log_{10}$ cfu (50 cfu) per 2.14 cm^2 of chicken skin.

Three MAR serotypes of *Salmonella* (i.e., Typhimurium, Kentucky and Hadar) were used in this study. All three strains occur in nature and all three strains are resistant to multiple antibiotics. Selective media with four antibiotics to which these strains were resistant to were used to allow their detection in the presence of native microflora. The effect of kosher salt and rinsing on prevalence of serotype Kentucky was similar to that of serotype Typhimurium, indicating that the beneficial effect of kosher salt on enhancing the removal of *Salmonella* was not specific to one serotype. However, persistence of the serotype Hadar on chicken skin following kosher salting and rinsing was significantly less than that of serotypes Typhimurium and Kentucky, suggesting that differences in persistence of *Salmonella* serotypes on chicken skin during kashering may exist in commercial plants.

CONCLUSIONS

A series of experiments that simulated the effect of kosher salt and rinsing on the persistence of *Salmonella* serotypes on chicken skin was conducted in the laboratory and led to the following specific conclusions:

(1) kosher salt by itself did not alter the persistence of *Salmonella* on chicken skin;
(2) kosher salt followed by rinsing reduced persistence of *Salmonella* on chicken skin to a greater extent than rinsing alone;
(3) the effect of kosher salt plus rinsing on persistence of *Salmonella* was similar at 12 and 24C; and
(4) differences in persistence were observed among *Salmonella* serotypes when chicken skin was exposed to kosher salt followed by rinsing.

Overall, these results indicate that kosher salting followed by rinsing is an important *Salmonella* reduction step in the kosher processing of poultry and that it may select for the persistence of specific serotypes of *Salmonella*.

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