Cross Contamination of Turkey Carcasses by *Salmonella* Species During Defeathering

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ABSTRACT *Salmonella* present on the feathers of live birds could be a source of contamination to carcass skin during defeathering. In this study, the possibility of transfer of *Salmonella* from the feathers of live turkeys to carcass tissue during the defeathering process at a commercial turkey processing plant was investigated. The contribution of scald water and the fingers of the picker machines to cross contamination were also examined. Over 4 visits, swab samples were collected from 174 randomly selected tagged birds before and after defeathering. Two swab samples from the fingers of the picker machines and a sample of scald water were also collected during each visit. Detection of *Salmonella* was carried out following standard cultural and identification methods. The DNA fingerprints obtained from pulsed field gel electrophoresis of *Salmonella* serotypes isolated before and after defeathering, from scald water, and from the fingers of the picker machines were compared to trace cross contamination routes. *Salmonella* prevalence was similar before and after defeathering during visits 2 and 3 and significantly increased after defeathering during visits 1 and 4. Over the 4 visits, all *Salmonella* subtypes obtained after defeathering were also isolated before defeathering. The results of this study suggest that *Salmonella* was transferred from the feathers to carcass skin during each visit. On each visit, the *Salmonella* subtypes isolated from the fingers of the picker machines were similar to subtypes isolated before and after defeathering, indicating that the fingers facilitate carcass cross contamination during defeathering. *Salmonella* isolated from scald water during visit 4 was related to isolates obtained before and after defeathering, suggesting that scald water is also a vehicle for cross contamination during defeathering. By using molecular subtyping, this study demonstrated the relationship between *Salmonella* present on the feathers of live turkeys and carcass skin after defeathering, suggesting that decontamination procedures applied to the external surfaces of live turkeys could reduce *Salmonella* cross contamination during defeathering.

Key words: *Salmonella*, turkey, defeathering, cross contamination

INTRODUCTION

*Salmonella* is one of the leading causes of bacterial foodborne illness in the United States, with an annual economic impact estimated at $0.5 to 2.3 billion (Frenzen et al., 1999). Poultry meat is commonly implicated in human salmonellosis (Morris and Wells, 1970), with broilers and turkeys reported as vehicles. Identifying sources of contamination during slaughter and processing and implementing strategies to reduce, eliminate, or prevent such contamination are key to reducing the prevalence of *Salmonella* on poultry and the consequent incidence of human salmonellosis. To date, most studies of *Salmonella* cross contamination during poultry processing have used broilers, and there is limited information relating to turkeys.

Birds presented for processing may harbor *Salmonella* in their feces but not show any symptoms of disease (Rigby and Petit, 1980, 1981; Higgins et al., 1982) and serve as potential contamination sources to uncontaminated birds.

Defeathering, evisceration, and chilling are processing stages where cross contamination may occur (James et al., 1992; Hafez et al., 1997; Ono and Yamamoto, 1999). Contaminated external surfaces such as feathers may have a direct contaminating effect on carcass skin during early processing stages, but there are few data documenting this effect. To date, no study has examined the relationship between *Salmonella* contamination of the feathers of live birds and defeathered carcasses.

The defeathering process, which consists of scalding, followed by mechanical feather removal, has been reported to be a site of significant microbial cross contami-
nation (Ono and Yamamoto, 1999). Possible mechanisms of bacterial cross contamination during defeathering include aerosols (Allen et al., 2003a,b), direct contact between contaminated and uncontaminated carcasses (Mulder et al., 1978), and the action of the fingers of the picker machines (Clouser et al., 1995a,b; Berrang et al., 2001; Allen et al., 2003a,b). Studies of cross contamination during defeathering have focused on the change in prevalence that occurs before and after defeathering and the dissemination pattern of an indicator organism from an artificially contaminated bird to other birds during processing (Clouser et al., 1995a,b; Allen et al., 2003a,b). To our knowledge, no study has examined the relationship between Salmonella subtypes isolated before and after defeathering.

Molecular typing is increasingly being used to complement conventional methodologies to elucidate bacterial transmission routes (De Cesare et al., 2001; Sander et al., 2001). Several studies have used molecular typing to understand the transmission of Salmonella within poultry production (De Cesare et al., 2001; Bailey et al., 2002; Liebana et al., 2002; Crespo et al., 2004). The application of such techniques will provide a better understanding of the mechanisms of cross contamination that may occur during defeathering.

In this study, the breast feathers of turkeys were sampled before defeathering and the exposed breast skin of carcasses after defeathering to determine the extent of cross contamination occurring during defeathering. Serotyping and pulsed field gel electrophoresis (PFGE) were used to more specifically determine the relationship between Salmonella isolates obtained pre- and postdefeathering.

MATERIALS AND METHODS

Description of the Defeathering System

This study was carried out in a commercial turkey processing plant that processes live turkeys to raw and cooked finished products. Turkeys were processed at an average speed of 800 carcasses/h. Birds were stunned, killed, and exsanguinated as they were navigated through a 20 to 30 ft tunnel. Carcasses were immersed in a scald tank that was continuously supplied with steam-heated water pumped through pipes at the bottom of the tanks. Samples were collected from each of 4 visits to the plant that were planned to coincide with the slaughter of flocks from farms with a history of Salmonella contamination. The temperature of scald tank water on the first 2 study days was 60°C and was 63.5°C during the third and fourth visits. The average immersion time per bird in the scald tank was 2.2 min. After scalding, the carcasses were conveyed to the picker machines, which consisted of 2 compartments. The first compartment was a Barker Gent-L-Flex picker with hock and straddle picker add-ons (Barker/Foodcraft, New Holland, PA). This compartment removes about 90% of all feathers. The second compartment was a Dura II-T (Simon Johnson, Barker/Foodcraft) picker, which removes the remaining feathers. The total time for feather removal for each carcass was about 2.2 min, bringing the total defeathering time to 4.4 min.

Sample Collection

To ensure that the same randomly selected birds were sampled before and after the defeathering process during each visit, brightly colored plastic zip-ties were interlocked on the metal link above the shackles holding the selected live birds. After the tagged carcasses emerged from the picker machine, a second set of samples was collected. Over 4 visits, 174 samples were collected at prededefeathering and 174 samples were collected at postdefeathering. Two swab samples were collected from the fingers of the picker machines, and 1 sample of scald water was collected during each visit.

Sampling Method

Commercially available sponges (Whirl-Pak SpecSponge, Nasco, Fort Atkinson, WI) moistened with 10-mL single strength buffered peptone water (BPW; Oxoid Ltd., Basingstoke, UK) were used for carcass and picker finger sampling.

For carcass sampling prior to defeathering, using a sterile gloved hand, the sponge was squeezed inside the bag to remove excess liquid before using it to swab an undeclared 100 cm² area on the breast feathers of randomly selected tagged live birds. Following defeathering, an undelimited 100 cm² area of the carcass breast tissue of the tagged defeathered bird was swabbed. Swabbing before and after defeathering was carried out 10 times vertically and 10 times horizontally using opposite sides of the sponges. For picker finger sampling, sponges were handled in the same manner as with carcass sampling and were used to swab an undelimited 200 cm² area of the picker machines that included the rubber fingers. After sampling, all swabs were returned to their original bags. Thirty milliliters of scald water was collected using a sterile water “dippa” sampler with an integral handle (Bibby Sterilin, Staffordshire, UK). All swab and scald water samples were stored in a chilled container and transported to the laboratory within 2 h of sample collection.

Microbiological Analysis

Forty milliliters of single strength BPW (Oxoid) was added to sponge samples and stomached for 90 s. For scald water samples, a 15-mL aliquot was added to 15 mL of double strength BPW (Oxoid). Sponge and scald water samples were incubated at 37°C for 18 to 24 h. Following incubation, aliquots of 0.5 and 0.1 mL of the BPW enriched samples were transferred to 10 mL of Tetrationate (Difco, Sparks, MD) and 10 mL of Rappaport Vassiliadis broth (Difco) and incubated at 42°C for 18 to 24 h. After incubation, a loopful of Tetrationate and Rappaport Vassiliadis broth were streaked onto XLT4
(Difco) and Brilliant Green Sulfa (Difco) agar plates. Plates were incubated at 37°C for 24 to 48 h. Up to 6 presumptive Salmonella colonies, when available, were picked from the selective agar plates and streaked onto Lysine Iron Agar (Difco) and Triple Sugar Iron (Difco) agar slants and incubated at 37°C for 18 to 24 h. Suspect Salmonella colonies following biochemical screening were confirmed using the Sensititre method for automated identification of gram-negative organisms (AP 80, Trek Diagnostics, Cleveland, OH). Confirmed Salmonella isolates were serotyped by the National Veterinary Services Laboratories, Ames, IA.

**Pulsed Field Gel Electrophoresis of Salmonella Isolates**

Identified Salmonella isolates were subtyped by PFGE using the standardized protocol described by the Centers for Disease Control and Prevention National Molecular Subtyping network for Foodborne Disease Surveillance (CDC, 2001). Salmonella Branderup H9812 (ATCC#: BAA-664) was used as the reference strain.

The DNA macrorestriction fragments were resolved on 1% SeaKem Gold Agarose (Cambrex Bio Science Rockland Inc., Rockland, ME) in 0.5x Tris-borate EDTA. Then PFGE was carried out in 0.5x Tris-borate EDTA using the Chef Mapper PFGE system (BioRad, Hercules, CA) with recirculation at 14°C. Run time for electrophoresis was 18 h with initial switch time of 2.16 s and final switch time of 63.8 s. Following electrophoresis, gels were stained in ethidium bromide for 30 min. Destaining was carried out in reagent grade water for 60 min. Images of macrorestriction patterns on agarose gels were captured using an imager (Alpha Innotech UV imager, San Leandro, CA) and stored as.tif files. Macrogen restriction patterns were compared using the BioNumerics Fingerprinting II Informatix software (Version 3.0, Applied Maths, Austin, TX; BioRad). The similarity index of the isolates was calculated using the Dice correlation coefficient option of the software with a position tolerance of 1% and an optimization of 5%. The unweighted-pair group method using average linkages (UPGMA; Struvelens, 1996) was used to construct dendrograms. A 70% similarity index was used to distinguish different clusters on the dendrograms.

**Statistical Analysis**

The McNemars test was performed using SAS 9 (SAS Institute Inc., 2004) to determine if there was a significant change in the prevalence of Salmonella-positive birds after defeathering for each visit. The McNemars test was also used to determine if there was a difference between the overall Salmonella prevalence before and after defeathering when all 4 visits were combined (Table 1).

### RESULTS

#### Change in the Prevalence of Salmonella-Positive Birds at Pre- and Postdefeathering

During visits 1 and 4, the Salmonella prevalence after defeathering was significantly higher ($P < 0.05$) than before defeathering (Table 1). There was no significant difference in Salmonella prevalence pre- and postdefeathering during visits 2 and 3. When all 4 visits were combined, there was an overall significant increase ($P < 0.05$) in Salmonella prevalence after defeathering.

#### PFGE Profiles

Dendrogram analysis revealed 4 clusters each for visits 1, 2, and 3, as well as 5 clusters for visit 4 (results not shown). During all visits, serotyping correlated with clustering because only identical serotypes were in the same cluster. Using visit 3 as an example, Figure 1 shows the dendrogram generated. Clusters 1 and 2 contain only S. Hadar isolates, whereas clusters 3 and 4 contain S. Muenster isolates.

#### Relationship Among Salmonella Subtypes Isolated at Pre- and Postdefeathering, from Scald Water and the Rubber Fingers of the Picker Machines

All Salmonella serotypes isolated after defeathering were also isolated before defeathering (Table 2). Salmonella serotypes with similar PFGE profiles were detected at pre- and postdefeathering and on the fingers of the picker machines during all 4 visits (Table 2). During visits 1 and 3, S. Hadar was isolated at the pre- and postdefeathering stages and on the fingers of the picker machines (Table 2). During visit 2, S. Schwarzengrund was isolated at pre- and postdefeathering and from the fingers of the picker machines (Table 2). During visit 4, S. Hadar was detected in scald water at pre- and postdefeathering and from the fingers of the picker machines (Table 2).

### DISCUSSION

The results of this study showed that there was transfer of Salmonella from live turkeys to carcass skin during defeathering. Cross contamination during defeathering...
Figure 1. Dendrogram showing pulsed field gel electrophoresis (PFGE) profiles of *Salmonella* isolates from visit 2. Pre = Predefeathering; Post = postdefeathering; Fingers = fingers of picker machines.
resulted in at least a similar number of birds being contaminated after defeathering as before (visits 2 and 3). On the other 2 occasions, there was a significant increase in contamination after defeathering as before (visits 2 and 3).

The results of the current study indicate that contamination during defeathering where conclusions were limited by a lack of subtyping data.

Characterizing isolates by serotyping and PFGE further facilitated the determination of the source and transmission route of Salmonella contamination during defeathering. This is in contrast to previous studies of cross contamination during defeathering where conclusions were limited by a lack of subtyping data.

This is the first report of a clear link between Salmonella contamination of turkey feathers and subsequent carcass contamination. Previous studies have determined the Salmonella status of live birds by sampling feces (Rigby and Petit, 1980, 1981; Higgins et al., 1982). In another study, the increase in Campylobacter contamination of broilers that occurred after defeathering was attributed to the expulsion of feces from the cloacae by the peristaltic action of the fingers of the picker machines (Berrang et al., 2001). The results of the current study indicate that contaminated feathers should also be considered as a source of carcass contamination. Given that such contamination is on the external surface of bird, it is reasonable to suggest that it could be a more significant source of carcass contamination than feces that is normally internal.

The detection of the same Salmonella subtype in scald water and at pre- and postdefeathering during visit 4 indicates that scald water is a vehicle for the transfer of Salmonella between birds. This is supported by another study in which Escherichia coli K12 was used as an indicator organism to demonstrate cross contamination during scalding (Mead et al., 1994). Despite the difference in the scalding temperature between visits 1 and 2 (60°C) and visits 3 and 4 (63.5°C), it is unlikely that Salmonella was absent in scald water during visit 1 and 2. Salmonella has previously been isolated from scald water at 60°C (Nivas et al., 1973). Collecting 1 sample of scald water per visit during the current study may have been a limiting factor for Salmonella detection. Parameters such as sample volumes, sample numbers, and culturing procedures may also influence Salmonella detection in scald water (Cason et al., 2000). Further studies that incorporate these variables will provide more information on the potential of scald water as a vehicle for cross contamination during processing.

The isolation of similar Salmonella subtypes from the fingers of the picker machines during all 4 visits (Table 2) and from birds at pre- and postdefeathering supports the role of the picker fingers in carcass cross contamination. This is in agreement with previous studies that have reported that the fingers of picker machines may facilitate bacterial cross contamination between carcasses (Clouser et al., 1995b; Berrang et al., 2001).

Neither scald water nor the fingers of the picker machines are cleaned nor replaced between carcasses, supporting their potential for facilitating cross contamination between Salmonella-contaminated birds and Salmonella-negative birds when they are processed in succession. Scald water and the fingers of the picker machines may also contribute to the contamination of Salmonella-free flocks when they are processed following a Salmonella-positive flock.

The results of this study show evidence for the possible transfer of Salmonella from turkey feathers to carcass skin during defeathering. This direct contaminating effect was greater during visits 1 and 4 when the Salmonella prevalence after defeathering increased significantly. Salmonella contamination on the turkeys feathers may, therefore, be a useful indicator of the potential for cross contamination during defeathering. Future strategies could focus on reducing the level of Salmonella on the feathers of live birds, thus minimizing the risk of cross contamination during defeathering.

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