Food Control 66 (2016) 250–255

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

The relationship between *Salmonella* levels in chicken spleen and mechanically separated ground chicken

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ARTICLE INFO

Article history:
Received 29 November 2015
Received in revised form
12 February 2016
Accepted 13 February 2016
Available online 17 February 2016

Keywords:
Salmonella levels
Spleen
Ground chicken
Most probable number

ABSTRACT

The objective of this study was to determine the relationship between *Salmonella* levels (presence and numbers) in chicken spleens and in mechanically separated chicken (MSC) at a commercial processing plant. Composite spleen and MSC samples were collected from 18 flocks at a commercial poultry processing plant. A total of 180 samples of each type were collected and tested for *Salmonella* using the most probable number (MPN) and enrichment methods. Overall, *Salmonella* was detected in 15.6% and 27.8% of spleen and MSC samples, respectively. The mean log MPN was 0.95 and 0.85 for the spleen and MSC samples, respectively. There was a significant relationship between *Salmonella* presence in spleen and MSC samples. However, the log MPN numbers in MSC samples were not significantly related to those in spleen samples. *Salmonella* presence in composite spleen samples (an indication of systemic infection in chickens) may predict MSC contamination with this pathogen, at the flock level.

1. Introduction

Chicken meat is a popular protein source in the United States (National Chicken Council, 2012). Americans consume more chicken than other types of meat; i.e., approximately 80 pounds per capita per year (United States Department of Agriculture (USDA), 2014a). The U.S. has the largest broiler chicken industry in the world (United States Department of Agriculture (USDA), 2014a). For instance, in 2014, 8.5 billion broiler chickens, weighing 51.3 billion pounds (live weight), were produced in the U.S. (United States Department of Agriculture (USDA), 2014c).

Salmonella spp. are among the major causes of foodborne disease in the U.S. and worldwide (Ao et al., 2015; Majowicz et al., 2010; Scallan et al., 2011; Voetsch et al., 2004). It is estimated that more than one million cases, 19,000 hospitalizations, and 370 deaths occur in the U.S. annually due to *Salmonella* spp. infections (Scallan et al., 2011). According to the CDC outbreak data (1998–2008), 10–29% of U.S. annual foodborne salmonellosis was associated with consumption of poultry meat (Painter et al., 2013). Five outbreaks caused by *Salmonella* linked to chicken products including one associated with mechanically separated chicken (MSC) product were reported between 2010 and 2014 (Centers for Disease Control and Prevention (CDC), 2010, 2011, 2013a, 2013b, 2014).

The overall *Salmonella* prevalence on chicken carcasses at the processing plants in the U.S. has been low mainly due to changes in management strategies and intervention practices over the past 20 years. In a recent USDA-Food Safety and Inspection Service (FSIS) annual report (2014) of *Salmonella* testing of raw meat and poultry products, *Salmonella* prevalence on young broilers was 3.8% (n = 8861); whereas, the prevalence in None Ready-to-Eat (NRTE) comminuted poultry was relatively high; i.e., 39.0% (n = 1789) and 82.9% (n = 2150) in ground chicken and MSC, respectively (United States Department of Agriculture (USDA), 2014b). Due to the concern with the reported prevalence in NRTE comminuted poultry, USDA-FSIS has recently increased the analytic portion for *Salmonella* testing from 25 g to 325 g (United States Department of Agriculture (USDA), 2015b). Furthermore, in 2015, FSIS has proposed a pathogen reduction performance standard for NRTE comminuted chicken; i.e., no more than 13 positives out of 52 samples (United States Department of Agriculture (USDA), 2015a).

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http://dx.doi.org/10.1016/j.foodcont.2016.02.021
0956-7135/

Chicken meat is primarily sold to retail as whole, parts, and as ground chicken. Ground chicken can be classified into two types of products: MSC and non-MSC ground product. Mechanically separated chicken is produced by grinding bone-in chicken parts like backs, necks, and wings. The crushed meat and bones are pushed through a metal screen that separates edible a minced (non-paste like) product from non-edible bone and cartilage product. On the other hand, non-MSC ground chicken is produced by grinding boneless skin-on/skinless parts such as drumstick, thigh, and breast. The chicken skin utilized in this process is a source of fat in the ground products.

When a *Salmonella*-positive chicken flock is processed and its parts grinded downstream, it can lead to ground chicken contamination. *Salmonella* association with chicken carcasses and parts can be external and/or internal (Bailey, Cox, Craven, & Cosby, 2002; Bailey et al., 2001; Berghaus et al., 2013; Cox et al., 2007; Kassem, Sanad, Stonerock, & Rajashekara, 2012; Northcutt, Berrang, Dickens, Fletcher, & Cox, 2003; Rivera-Pérez, Barquero-Calvo, & Zamora-Sanabria, 2014; Velaudapillai, 1964; Volkova et al., 2010; Wu, Alali, Harrison, & Hofacre, 2014). While external skin contamination may predict presence of *Salmonella* in ground poultry as shown in a previous study conducted at a turkey processing plant (Cui, Guran, Harrison, Hofacre, & Alali, 2015), there is no study in the published literature that examined the relationship between *Salmonella* levels (presence and numbers) in chicken internal organs such as spleen and post-harvest products (e.g., ground chicken). It has been revealed that *Salmonella* can be internalized in poultry internal organs and parts, for example, in spleen, liver, and bones (Cox et al., 2007; Cui et al., 2015; Kassem et al., 2012; Velaudapillai, 1964; Wu et al., 2014). Spleen has been shown to harbor *Salmonella* more frequently than other organs (Hafez, Stadler, & Kösters, 1997; Rostagno, Wesley, Trampel, & Hurd, 2006). We hypothesized that presence and numbers of *Salmonella* in spleen may indicate highly contaminated flocks and consequently higher levels of ground chicken contamination. Therefore, the objective of this study was to determine the relationship between *Salmonella* levels (presence and numbers) in chicken spleens and in MSC ground product at a commercial processing plant.

2. Materials and methods

2.1. Sample collection

This cross-sectional study was carried out between August 2013 and February 2014 in cooperation with a commercial poultry plant in Northeast Georgia, USA. Spleen and MSC samples were collected from 18 flocks at the processing plant. Two to three chicken flocks were sampled every month over the study period. The flocks included in this study were those processed during the night shift (i.e., 11 p.m.–2 a.m.). Only one flock was processed during that shift at the processing plant. This is to ensure that MSC and spleens samples were from the same flock.

During each visit to the plant and at the start of processing a flock, 10 composite spleen samples (approximately 30 g each) and 10 MSC samples (25 g each) were collected. The spleen composite samples were gathered right after the USDA-FSIS inspection of eviscerated carcasses. The sample collection was as follows: Ten chicken viscera from 10 consecutive carcasses were removed off the processing line. Thereafter, 10 spleens were harvested from the viscera using a cutting scissors. The 10 spleens representing 10 carcasses were placed in one Whirl-Pak bag (NASCO, Fort Artkinson, WI) forming one composite sample. Scissors were sanitized with 70% ethanol between each composite sample. The 10 composite spleen samples were collected over 100 min (i.e., one composite sample every 10 min). As for the MSC samples, they were collected at the outlet of the grinding machine (one ~25 g sample every 5 min). This MSC product was produced by grinding chicken frames that consisted of bones, meat, cartilage, skin, and fat. The grinder then separated the finished product from the bones and cartilage. The product had a minced product texture, not a paste-like or batter-like consistency. All samples were immediately transported on ice to the laboratory at the Center for Food Safety, University of Georgia (Griffin, GA) for further process and *Salmonella* analysis.

2.2. Salmonella analysis of composite spleen and ground chicken samples

Upon arrival to the laboratory, the spleens were sterilized via immersion in boiling water for 5 s and then transferred aseptically to a new sterile blending bag (NACSO, Fort Artkinson, WI). The purpose of this sterilization step was to kill *Salmonella* on the spleen surface without damaging the organism cells in the spleen (if present). This sterilization procedure was based on our preliminary studies (data not shown). The spleens inside the bag were smashed manually by hands then 300 ml of buffered peptone water (BPW; Difco, Becton Dickenson, Sparks, MD) was added to the bag. The sample bag was then stomached at high speed for 2 min (Stomacher 400, Seward Ltd, London, England).

2.2.1. Most probable number method to quantify Salmonella

A 3-tube 3-dilution most probable number (MPN) analysis was conducted to quantify *Salmonella* numbers in the samples. For each sample solution, nine tubes were used for the pre-enrichment of *Salmonella* with the first three tube-set containing 10 ml of the original sample solution. The second and third sets of three tubes contained 1 ml sample solution (plus 9 ml BPW) and 0.1 ml sample solution (plus 9.9 ml BPW), respectively. The nine tubes were incubated at 37 °C for 24 h. A 0.5 ml aliquot of the BPW was transferred to 10 ml tetrathionate broth (TT; Difco) and then incubated (42 °C, 24 h). After incubation, a loopful of the TT broth was streaked on xylose lysine tergitol-4 (XLT-4; Difco) agar plates and incubated (37 °C, 24 h). Up to three presumptive black *Salmonella* colonies were transferred to triple sugar iron agar (TSI; Difco) slants and lysine iron agar (LIA; Difco) slants and incubated (37 °C, 24 h). Isolates with typical *Salmonella* reactions on TSI and LIA were then confirmed by the agglutination *Salmonella* Poly O A-I & Vi antiserum test (Difco). The numbers of positive tubes were recorded for each sample and the MPN/g value of each sample was retrieved using the USDA-FSIS MPN table (United States Department of Agriculture (USDA), 2008).

The MSC samples (25 g) were transferred aseptically into new sterile blending bags. A 225 ml of BPW was added to each bag and then the solution was stomached at high speed for 2 min. *Salmonella* quantification of MSC samples via MPN method was conducted similarly to the spleen samples.

2.2.2. Primary and delayed secondary enrichments for Salmonella detection

In addition to *Salmonella* quantification, we enriched the remaining sample solutions to detect low levels of this organism (i.e., primary enrichment) undetectable via the MPN method. A 30 and 25 ml of 11X TT broth were added to the original spleen and MSC sample solution, respectively, and incubated (42 °C, 24 h). On the next day, a loopful of the sample solution was streaked on a XLT-4 plate. The plates were then incubated at 37 °C for 24 h. The remaining isolation and confirmation of *Salmonella* was done as described for MPN.

In conjunction with the primary enrichment of the samples, a