TITLE: Production Practices and Prevalence of *Salmonella* on Chicken Carcasses Processed in Facilities Exempt from the Federal Poultry Products Inspection Act

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SUMMARY: Microbiological data for the prevalence of *Salmonella* spp. in poultry processed in facilities that operate under exemption from continuous inspection is lacking on state and national levels. This data is essential to identify best practices to reduce risk of foodborne illness. The primary purpose of this study was to characterize the poultry processing operations and understand prevalence and spread of *Salmonella* spp. during slaughter. Oregon’s exempt poultry facilities (n=16) were asked to participate in a *Salmonella* testing study. Five agreeable processors were randomly selected, and dressed carcasses (n = 15-16) were tested for *Salmonella* using a modified USDA-MLG method. Two facilities were selected for further environmental and carcass sampling. The initial round of carcass testing revealed substantially different *Salmonella* prevalence: 3 processors: 0 detectable *Salmonella* (n=15); 1 processor: 1/15 positive; 1 processor: 10/16 positive. The facility with highest prevalence (A) and one facility with no detectable *Salmonella* (B) were selected for sampling during active processing. Facility A had very high rates of *Salmonella* in live birds (16/30 positive feces; 17/20 positive crops). Facility A also had positive carcasses (16/20), equipment/tools (6/7), and processing water (9/24). For facility B, positive samples were only detected in fecal samples (2/30) and a single crop (n=20). All other samples from B [carcasses (n=20), equipment/tools (n=6), processing water (n=33)] were negative for *Salmonella*. *Salmonella* prevalence in poultry from Oregon’s exempt poultry processors ranged from very high (>60%) to very low (0/15). The processing facility that produced carcasses with no detectable *Salmonella* had few birds carrying *Salmonella*, but processing practices were successful at preventing spread to carcasses. The facility with high rates of *Salmonella* in carcasses also had an exceptionally high rate in live birds, which was not mitigated by processing. On-farm production practices should be evaluated to identify best practices to mediate the spread of *Salmonella* in live birds.

OBJECTIVES:

1) Characterize common practices of state-licensed poultry processing facilities and identify potential areas for intervention to improve safety.

2) Determine preliminary *Salmonella*-prevalence rates in poultry processed in facilities exempt from federal inspection.

3) Evaluate poultry processing environments during slaughter to identify practices that might increase or reduce *Salmonella* rates on finished carcasses.
PROCEDURES:

1.1 Poultry production and processing questionnaire
A five-page questionnaire was developed to characterize poultry production and processing practices of Oregon’s state-licensed poultry processors. The questionnaire was composed of five primary sections: general business information; poultry production; slaughter and processing practices; facilities, hygiene, and disinfection practices; testing. Most questions were short answer with some answers requiring selection from a defined set of possible answers or a check box yes/no. The survey was submitted to Oregon State University’s Institutional Review Board and was deemed to not fit the definition of human subjects research. A list of state-licensed poultry processing facilities (17) was obtained from the Oregon Department of Agricultures Food Safety License database (www.oda.state.or.us/dbs/licenses/Pages/default.aspx). The questionnaire, a cover letter, and postage-paid return envelope were sent by standard mail in May 2013 to the processor’s address on file requesting the return of the completed survey within two weeks. A second questionnaire was sent in July 2013 to those that had not responded to the initial request. Data from the completed questionnaires were entered into a spreadsheet (Microsoft Excel) for collation and analysis.

1.2 Initial carcass testing
Five survey respondents that had agreed to participate in further testing were randomly selected and contacted to coordinate the purchase of 15-16 fresh (never frozen) poultry carcasses in the summer of 2013. For four of these facilities, carcasses were purchased from the processor at a farmers’ market and transported to the laboratory in a cooler sufficiently packed with ice to maintain ≤4°C. The remaining processor delivered the carcasses to the laboratory. All carcasses were held at 4°C from receipt until analysis (24-72 h).

Carcasses were analyzed for the presence of Salmonella following the standard USDA Microbiology Laboratory Guidebook (USDA-FSIS, 2011) with a few modifications. Suspect colonies (3 per sample) were transferred to CHROMagar Salmonella Plus (DRG International) and incubated at 37°C for 24-48 h. Isolates displaying mauve/pink/magenta coloration were transferred to Tryptic Soy Broth (TSB; Neogen) and incubated at 37°C for 24 h. Isolates were confirmed as Salmonella spp. using the MicroSeq Salmonella real-time PCR assay (Life Technologies, Carlsbad, CA) and the API20E (bioMerieux, France).

1.3 Environmental sampling of processing facilities
Based on the results of the initial carcass testing, two facilities were selected for further observation and sample analysis of their processing facility in the summer of 2014. The facilities were toured, slaughter was observed, and operations were diagramed to identify appropriate sampling protocols that would cause minimal inconvenience to the processing personnel. Environmental samples of machinery, tools, and surfaces of the processing facility were taken of a 15 cm x 15 cm area using a Quick Swab (3M, St. Paul, MN). Samples from the same areas were collected prior to processing, after processing, and after cleaning. Water samples (5 mL) from all water tanks were collected every 20 minutes throughout processing in both facilities. Blood samples (5 mL) were collected from the catch basin beneath the kill cones. Fecal samples (3 g) were collected from the transport crates. Closely matched samples were collected at the two processing facilities. All environmental samples were held on ice (≤4°C) immediately after collection through transport back to the laboratory. Sample analysis was initiated upon arrival to the laboratory. Swab samples
were enriched in 10 ml BPW. Water samples (5 ml) were enriched with 2x BPW (5 ml). Blood (1 g) and fecal samples (3 g) were enriched in BPW (1:10). *Salmonella* analysis was performed as described above.

1.4 Carcass and crop testing
At both facilities, carcasses and their respective crops were collected directly from the evisceration table into individual Whirl-Pak bags. Eight random birds were analyzed, along with the first and the last of the processing session. An additional ten carcasses (with matching crops) were collected from the chill tank after completion of the processing session. These chickens were selected at the evisceration table, the crop was collected, and the carcass was tagged with a unique tag to identify itself for collection after chilling. All crop and carcass samples were held on ice (≤4°C) immediately after collection through transport back to the laboratory. Carcass samples were analyzed as described above. Crop samples were hand massaged with 100 mL BPW. *Salmonella* analysis was performed as described above.

SIGNIFICANT ACCOMPLISHMENTS:
- Small poultry processing and production practices were characterized through the use of a survey instrument.
- Preliminary prevalence rates of small processing operations were determined and appeared to demonstrate a bi-modal distribution of prevalence (i.e., <10% and >50%).
- Preliminary prevalence rates were confirmed with a second sampling the following production season.
- The prevalence of *Salmonella* in environmental samples and carcasses at the time of slaughter is most directly impacted by the relative *Salmonella* prevalence on the live birds. Processing practices can contribute to the spread; however, on-farm production practices play a larger role. This is a very important result that will influence not only future research but also outreach and education targeting this growing sector of Oregon agriculture.

BENEFITS & IMPACT:
- An abstract focused on this research has been submitted for presentation at the International Association for Food Protection Annual Meeting that will be in Portland, OR in July 2015.
- A manuscript is in preparation for submission to the Journal of Food Protection or Food Protection Trends later this spring.
- An extension publication, to be prepared later this year, will explain the research findings to a lay audience and provide “best practice” guidance for small-scale, commercial poultry producers and small, exempt poultry processors. We will assure that the publication has wide distribution within and outside Oregon by doing outreach through the OSU Small Farms Program (and organization/agency partners) and the national Niche Meat Processor Assistance Network (directed by PI Gwin).
- Through this project, four undergraduate researchers received training in applied research related to food microbiology as well as opportunities to experience poultry slaughter and interact with processing personnel.
- The findings of this study emphasize the need to minimize food safety risks during the production of poultry. We are actively engaged with the owners of poultry operations to begin a study to identify production practices that may contribute to increased rates of *Salmonella* in live birds. In doing so, we aim to identify best practices throughout the
poultry production cycle to reduce prevalence of *Salmonella* in both live birds and finished carcasses. Until now, more focus has been placed on preventing contamination during processing in processing facilities. While this remains important, we have demonstrated that on-farm practices are as or more important. Interventions that address this will reduce risk for farmers, processors, marketers (retailers, restaurants), and consumers.

**ADDITIONAL FUNDING RECEIVED:** None currently.

**FUTURE FUNDING:** Looking for resources to continue on-farm testing to determine sources/niche of *Salmonella* on-farm. Also, exploring funding opportunities for sequencing persistent *Salmonella* strains compared to non-persistent strains to identify characteristics that contribute to persistence.