DISSEMINATION OF *SALMONELLA* SPP. IN A LARGE POULTRY SLAUGHTER PLANT

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Introduction

Poultry and its derivated products are considered one of the most important food sources to act as a vehicle for foodborne *Salmonella* (Bean and Griffin, 1990). Therefore, the control of this microorganism in poultry has been the focus of numerous reports worldwide. Several studies have shown that live birds can introduce this pathogen into the slaughter plant and be an important source of contamination of the final product (Fuzihara *et al.*, 2000; Boscan *et al.*, 2005). Additionally, the number of *Salmonella* strains with resistant to antibiotics has increased (Threlfall, 1992) in human and farm animals, becoming an important public health concern. In Venezuela, there is little information on how *Salmonella* spread throughout the slaughter and packing process and their antimicrobial susceptibility. The main objective of this research was to study the spread of *Salmonella* spp. in a large poultry slaughter plant by sampling carcasses at different stages during the processing, as well as edible by-products and the environment. Additionally, tests of antimicrobial susceptibility of the strains isolated were conducted.

Materials and Methods

Location and samples: The present study was performed in a large poultry slaughter plant located in Zulia State, Venezuela. Eighty five (n=85) birds approximately 6 week-old were randomly selected at their arrival to the plant to be tagged and followed through of the slaughter process. A total of 332 carcass samples were colleted in four different phases: carcasses after defeathering (DEF) (n=85), evisceration (EVIS) (n=85), chilling (CHI)(n=81), and final packed product (FPP)(n=81). Intestines (duodenal and colon) and internal organs (livers and spleens) of each selected bird (n=85) were also collected. Additionally, thirty five (n=35) samples from edible by-products such giblet pack (neck, n=9; liver, n=9; gizzard, n=9) and legs, n=8; as well as 103 environmental samples such as water, ice, and diverse surfaces were also collected.

Sampling procedure: Intestine and organs: These were collected by the researchers immediately after hocking and opening the carcasses using sterile gloves and placing the sample into prelabed sterile bags. Carcasses and packed product: Immediately after DEF, EVIS and CHI, the tagged carcasses were removed from the production line to be sampled. Each individual carcass was introduced in a sterile plastic bag containing 225 ml of Peptone Water. Once the bag was properly sealed, it was vigorously shaken and manually massaged for 1 minute. Then, the carcass was placed again in the production line. A similar process was performed with the FPP. Edible by-products: Necks, livers and gizzards from other birds that were being processed at the same time with the tagged birds, were pooled and collected in individual sterile bags. Eight legs from the storage room (during the process) were chosen and pooled into sterile plastic bags containing 225 ml of Peptone Water. Environmental samples: Surface samples from conveyor belts in the evisceration and packing room, chiller's slide (2 mt^2), hooks (total surface) from the evisceration and packing room, and baskets used in the packing process (50 cm^2) were collected throughout the slaughter process. Sterile swabs were used for sampling hooks and baskets, and for larger surfaces, such as the conveyor belts and chiller, sterile gauze pads were used. Water and ice samples: Using sterile flasks, 100 ml of potable water (used during processing), water from scalding/washing area, water from the chiller, and ice (commercially and in-plant produced for the chiller) were individually collected.

Microbiological analysis: Bacteriological methods recommended by the Food and Drug Administration were used (FDA, 2003) for *Salmonella* detection in the peptone water from carcasses and legs rinses, blending from edible subproducts and the environment. For the detection of *Salmonella* spp in the intestine and internal organs (liver and spleen) the bacteriological methods described by Animal Disease Diagnostic Laboratory, Ohio Department of Agriculture (2000) were used. For antimicrobial susceptibility testing, agar diffusion methods of Kirby and Bauer were used (Bauer *et al.*, 1996). Statistical analysis: Chi-squared analysis was used to test differences among frequencies for each slaughter process, edible by-products, environmental samples, water, ice samples and antimicrobial susceptibilities (SAS, 1996).

Results and Discussion

Forty five percent (38/85) of the intestine and internal organs sampled were positive to *Salmonella* spp. Therefore, almost half of the selected birds were already positive to *Salmonella* at their arrival to the plant. When carcass samples from the different phases of the process were analysed, 50.6% (168/332) were positive and distributed as follows: DEF 46/85 (54.1%), EVIS 37/85 (43.5%), CHI 43/81 (53%), and in FPP 42/81 (51.9%). No significant differences were observed (P=0.3). Further analysis revealed that from the initial 46 carcasses with positive surface contamination after the scalding and defeathering, 61% (28/46) were still positive at packing. Indeed, 43.6% (17/39) of the initial 39 carcasses negative after scalding and defeathering were contaminated at packing. This could indicate that the carcasses that initiate the slaughter process with surface contamination will be 2.01 (OR) times more likely to be contaminated at the end of the process (packed product). However, because the sample size was small, it was not possible to find a significant difference between such values (P =

0,11). When analyzing the possibility that infected birds (positive intestinal content for *Salmonella* at arrival) will be more likely to end as a contaminated packaged product, it was observed that from the 38 positive birds at arrival, 55% (21/38) of them ended up positive at packing, compared to 44% (21/47) of those birds that were negative at arrival but ended up positive at packing. This result may also suggest that infected birds are more likely (OR 1.53) to end as a contaminated product than negative birds at arrival; however, the difference between groups was not significant (P =0.33). Thirty four percent (n=12) of the 35 samples from edible subproducts (giblet pack and legs), were positive for *Salmonella*, and were distributed as follows: neck 4/9 (44.4%), liver 2/9 (22.2%), gizzard 3/9 (33.3%), and legs 3/8 (37.5%). No significant differences were observed (P= 0,8) among this group of samples. These results point out the widespread presence of *Salmonella* in the different edible by-products. With regard to the environmental samples, from the 103 collected samples, 15 (14.6%) were positive and distributed as follows: conveyor belt in the evisceration room, 1/9 (11.1%); conveyor belt in the packing room, 1/8 (12.5%); Chiller's slide 1/10 (10%); hooks at the evisceration room, 1/9 (11.1%); hooks at the packing room, 2/8 (25%); baskets for packing (beginning), 2/9 (22.2%); baskets for packing (end), 4/9 (44.4%); scalding water , 2/8 (25%); chiller's water, 0/9 (0%); potable water, 0/9 (0%); ice (commercial source) for chiller, 0/6 (0%); ice (in-plant source) for chiller, 1/9 (11.1%). The results highlight the presence of *Salmonella* in the environment, which could be an important source of contamination. With reference to antimicrobial susceptibility tests, the following results were obtained: 7.4% resistance to chloramphenicol (11/148), 10.8% to ciprofloxacin (16/148), 54.1% to neomycin (80/148), 84.5% to nalidixic acid (125/148), 62.2% to nitrofurantoin (92/148), 73% to tetracycline (108/148) and 53.4% to trimethoprim (79/148).

Conclusions

The high initial prevalence of infected poultry, the occurence of environmental contamination, and the possibility of cross contamination of the final product with *Salmonella* positive giblet packs are major factors that could explain the high number of contaminated packaged product, which is a major public health concern. Although it was expected to observe an increase in carcasses positive for *Salmonella* as they went through the process, especially in the last step (packing), in this study such an increase was not observed and the total prevalence of contaminated carcasses was steady through the four phases analysed. A possible explanation was the use of chloride (at least 20 ppm) in the chiller water, which could decrease the dissemination of *Salmonella*. Nevertheless, active dissemination and cross contamination of *Salmonella* did occur throughout the slaughter process, because some animals or carcasses that were negative at the beginning of the process were positive at the end of it. In this study it was not possible to show the association between infected poultry (or poultry with surface contamination) at arrival and the increase in the likelihood that the final product from those animals will be positive for *Salmonella*. It was, however, possible to observe a trend indicating that this could be a major risk factor; and therefore, possibly an important point of intervention. Additionally, a high level of resistance was found against antimicrobials of common use in humans, which also represents an important public health concern.

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