Optimizing Sampling Plans for Identifying Sources of *Salmonella*: An Example from a Multi-State Turkey Processing Plant Study

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ABSTRACT

Salmonellosis and listeriosis are two of the leading diseases that are involved with food borne illness outbreaks. Both organisms can persist in a poultry processing environment and contaminate finished product if programs are not in place to limit their introduction and control their spread once introduced. Most processors conduct weekly microbial environmental testing as a check of their control methods. The positive or negative results from each sampling set are indicators of the current levels of microbial contamination, but an ongoing compilation of the results can provide a wide variety of other information. An example set of data was generated from environmental samples collected in poultry processing plants in five states that were analyzed for *Salmonella* and *Listeria* species, among other pathogens. A total of 1,363 samples were analyzed for both organisms and another 66 samples were analyzed for *Salmonella* alone (1,429 total). Of these, 284 (19.9%) were positive for Salmonella, 264 (19.4%) were positive for *Listeria spp*., and 54 (4%) were positive for both organisms. The data was compiled in Microsoft Excel and the PivotTable function was used to generate summaries and analyze the data for trends. These methods can provide valuable information for optimizing an environmental sampling plan to increase efficiency at finding positive results and decrease costs.

KEYWORDS

Salmonella, *Listeria*, environmental sampling, poultry processing, turkey

1. INTRODUCTION

Microbiological sampling of the plant environment is an important tool that food processors use to determine the effectiveness of their sanitation practices and processes in controlling pathogens, including *Salmonella and Listeria* species. Evaluation of the test results over an extended period of time can illustrate changes that should be made in test sample quantities, types, frequency, time, location or analysis. The PivotTable function in Microsoft Excel provides one method of summarizing the data sets that result from long-term sampling and analyses. The charts and graphics that can be generated with this tool are particularly useful to identify trends and deficiencies over time. It is important to note that no one sampling program is suitable for all processing operations, and that even when an appropriate program is established changing conditions often necessitate updates and modifications. Meat and poultry processors must select the environmental sample types and schedules that will provide them with sufficient information to improve or maintain plant hygiene and to reduce the presence of pathogens. Common sample types include raw product or ingredients, equipment surfaces, processing water, floors, drains, and air. Pertinent information must be recorded for each sample that details type, time, date, location, and any other information that is deemed to be valuable for interpreting results. Microbiological samples can be analyzed for specific pathogenic organisms such as *Listeria monocytogenes,* indicator organisms such as generic *E. coli*, or organisms such as yeasts and molds that contribute to spoilage. A variety of qualitative or quantitative analyses are available, and the processor should choose the methods that produce the most valuable information for their plant. Regardless of the methods, results must be accurately reported and recorded.

Several factors must be accounted for in selecting the frequency and schedule of environmental sample collection. Some key factors are traffic patterns of product and employees within the plant, production volume, standard sanitation operating procedures that are in place, previous history of sample analysis data, and microbiological guidelines or action levels from the USDA Food Safety and Inspection Service (FSIS). The sample collection process can be based on a set schedule of type and location or random selections from a list or pool.

The FSIS requires all establishments that produce ready-to-eat meat and poultry

products to conduct *Listeria spp.* testing of food-contact surfaces between the lethality treatment (cooking step) and final packaging. Surfaces that could be sampled include conveyor belts, tabletops, slicing equipment, chill water or brines, and any other surfaces that product is in direct contact with along a processing line.

Environmental sampling can be costly in time and resources. Scott, et. al. (2005) provide examples of testing programs that include 10-15 weekly samples and an allowance for random re-testing of certain areas or testing of product samples in response to positive results. These example programs have an annual cost of $21,000-26,500, for supplies and analyses alone. An optimized sampling plan can limit costs by reducing the number of samples that must be collected to be confident in finding microbial contamination if any is present.

These analyses can be used for other data sets to determine which combinations of sample location, type, quantity, and frequency will optimize identification of target microorganism(s) and niches that can be hot spots for contamination of products. Regularly modified environmental sampling plans that can identify and predict the presence of *Salmonella* and *Listeria* species are vital in preventing food borne illnesses.

2. METHODS

2.1 SELECTION, COLLECTION, AND ANALYSIS OF SAMPLES

Environmental and raw product samples were collected from commercial turkey slaughter plants in five states. Sample collection sites were randomly selected in an attempt to provide a comprehensive view of the microorganisms present throughout each plant. Swab samples were collected with sterile sponge sample kits, which have been shown in research to be one of the most effective methods for qualitative analyses (Kovacevic, 2009). Product samples were collected aseptically and placed in sterile sample bags. Microbial species were isolated following USDA-FSIS isolation procedures (Johnson, 1998; Sparling, et. al., 2004). Samples were analyzed qualitatively for presence of *Salmonella* and *Listeria* species. *Listeria* isolates were further identified to species.

2.2 ORGANIZATION OF RESULTS

This study focuses on the qualitative (i.e., positive or negative) results of sample analyses, which are typically the focus of in-plant environmental sampling plans. A spreadsheet was created in Microsoft Excel 2007 that lists an identification number, sample date, isolation date, and brief description for each sample. Columns were created in the spreadsheet to classify the samples in a number of different ways. The samples were sorted into seven sample types: drains; walls and floors; product contact surfaces and equipment; process/chilling water; workers, gloves, and boots; post-chill raw product; and fecal contamination. They were also assigned to one of four areas based on the location of collection: transport through defeathering; evisceration; chilling; post-chill cut-up/packaging. Other categories that were used were season, state, and plant. The model sampling plans presented by Scott, et. al. (2005), use a classification system that divides samples into four zones based on relative potential risk for finished product contamination (e.g. product contact surfaces in the finished product area are in Zone 1, while the walls of a raw materials storage cooler are in Zone 4). The variables in each category were each assigned a letter. In the row for each sample, the letter and variable were listed under the category in each column. A one for a positive or a zero for a negative was placed in the columns for the final categories, *Salmonella, Listeria, or Salmonella* and *Listeria* results. For example, the row for a swab from a conveyor belt at trim up in plant A which was collected on May 18th and is positive for *Salmonella* but negative for *Listeria* reads: Spring, VA, A, D. Pack, C. Equipment, 1, 0, 0.

2.3 CALCULATIONS

The PivotTable function performs count, sum, and other basic functions as part of generating the tables. Other Excel formula functions were used to produce percentages and the results of the statistical analyses. The samples needed to find one positive result at a 95% confidence level were calculated with the formula: $n = -\ln(1-Cl)/p$; where n is the number of samples needed, Cl is the desired confidence level, and p is the proportion of positives out of the samples collected.

3. PivotTables

First, all of the data in the spreadsheet was selected using the "select all" function. Next, the PivotTable function was selected from the "Insert" tab. If only a portion of the data is desired, a range can be specified in the window that opens. It is advisable to place the table or chart in a new worksheet that is separate from the raw data. A single spreadsheet would be cluttered with figures in addition to the large amount of data generated through extended periods of routine sampling.

The PivotTable Field List displays the sorting categories that are column headers on the data spreadsheet. The fields can be dragged down into one of four areas for various sorting and comparative options. The field with the label of the results column should be placed in the Values section. Once a field is in one of the four areas, it can be clicked on to open a drop down menu. From this menu, the Value Field Settings can be used to display the results as a count of the number of samples in the category, a sum of the positives, or with other calculations. The fields that are desired for comparison can be dragged into either the Row Labels or Column Labels areas. Table 1 shows an example that was generated with "sample type" as the Row Label, "plant area" as the Column Label, and "sum of *Salmonella* positives" as the Value. This provides a summary of the positive results found for each sample type in each area of the plant. The PivotTables can be manipulated in various ways and used for further calculations. Table 2 shows the data from Table 1 displayed in a slightly different configuration and as percentages of positive samples instead of positive sample count.

4. RESULTS

A total of 1,429 samples were collected and analyzed for this study. The results of some of the analyses are shown in Tables 1 and 2. Of these, 1,363 were analyzed for both *Salmonella* and *Listeria* species, and 66 were analyzed for *Salmonella* alone. There were a total of 284 *Salmonella* positives (19.9%), 264 *Listeria* positives (19.4%), and 54 samples (4%) were positive for both. Table 2 is a PivotTable summary of the results, with added percentages. *Salmonella* was most prevalent in samples of fecal contamination (33.7% of samples), while *Listeria* was most prevalent in samples from drains (57.2% of samples). Interestingly, samples that were

taken from equipment used by personnel such as gloves, boots, and aprons had the highest percentage of positives for both organisms aside from drains (3.1% of samples). There was a steady decrease in prevalence of *Salmonella* as samples progressed through the processing environment, with 36.4% positive samples in transport, scalding, and defeathering locations, and only 11.2% in post-chill cutup, grinding, and packaging areas. The presence of *Listeria* species was more sporadic and did not appear to follow a trend. *Salmonella* was more prevalent in Summer and Fall than in Spring or Winter, as shown in Table 3.

5. DISCUSSION

The results of the environmental sampling data that were summarized in the PivotTables were used to evaluate the effectiveness of the sampling plan of a theoretical processing plant with the given results. The minimum numbers of samples needed to obtain a positive result for each plant area and sample type are shown in Table 4. The shaded areas denote the area or type with the lowest number necessary. Samples that are positive for both *Salmonella* and *Listeria* are least likely to be found on equipment and other product contact surfaces, especially in post-evisceration through water chilling areas (348 samples needed). Overall, samples taken in the transport, scalding, and defeathering areas are most likely to yield a *Salmonella* positive (8 samples needed).

6. CONCLUSIONS

The summary data from the PivotTables were used to create data that can be used to optimize environmental sampling. This information can be used to ensure that samples are taken in areas that are most likely to yield positive results, if the organism is present. Samples taken from areas and types that are least likely to yield positive results should be decreased. The data can be re-evaluated on a regular basis to ensure that the sampling plan is up to date with the demands of current conditions. The result is a plan that allows for fewer samples to be taken, thus saving the company money, while the likelihood of finding positive results is not affected.

7. REFERENCES

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TABLE 1. Sum of *Salmonella* positives for each sample type in each plant area.

Sum of *Salmonella*	**Column Labels**				
Row Labels	**A. Trans-Defeath**	**B. Evisc**	**C. Salt-Chill**	**D. Pack**	**Grand Total**
A. Drains	15	29	4	2	50
B. Structure	10	2	0	1	13
C. Equipment	66	21	9	7	103
D. Water	NA	0	35	3	38
E. Personnel	0	0	2	1	3
F. Product	NA	2	14	31	47
G. Fecal	1	29	NA	NA	30
Grand Total	**92**	**83**	**64**	**45**	**284**

NA: none analyzed.

TABLE 2. Percentage of *Salmonella* and *Listeria* positives for each plant area and sample type.

		Sample Type							
Plant Area		**Drains**	**Walls, Floors, Fixtures**	**Equipment, Product Contact Surfaces**	**Process or Chilling Water**	**Personnel, Gloves, Boots, Aprons**	**Raw Product: Carcass, Parts, Ground**	**Fecal Contamination**	**TOTAL for Plant Area**
Transport, Scalding, Defeathering	*Salmonella* positive	53.6%	17.9%	41.3%	NA	0.0%	NA	12.5%	36.4%
	Salmonella & *Listeria* positive	38.5%	3.6%	3.4%	NA	0.0%	NA	0.0%	7.2%
	Listeria positive	57.7%	17.9%	12.4%	NA	0.0%	NA	37.5%	19.5%
Evisceration, Gizzard Harvest, Reprocessing	*Salmonella* positive	41.4%	2.8%	18.1%	0.0%	0.0%	7.7%	35.8%	22.0%
	Salmonella & *Listeria* positive	31.7%	0.0%	2.7%	0.0%	0.0%	0.0%	2.8%	7.1%
	Listeria positive	54.0%	19.7%	9.9%	0.0%	16.7%	14.3%	12.7%	20.9%
Post-evisceration through Water Chilling	*Salmonella* positive	13.3%	0.0%	7.6%	28.9%	8.3%	34.1%	NA	16.2%
	Salmonella & *Listeria* positive	6.7%	0.0%	0.9%	1.8%	8.3%	0.0%	NA	1.8%
	Listeria positive	53.3%	35.5%	13.8%	3.6%	12.5%	7.3%	NA	16.6%
Post-chill Cut-up, Grind, Packaging	*Salmonella* positive	5.0%	1.4%	8.4%	25.0%	3.7%	18.3%	NA	11.2%
	Salmonella & *Listeria* positive	2.5%	1.4%	1.2%	0.0%	0.0%	1.3%	NA	1.3%
	Listeria positive	65.0%	29.2%	13.3%	0.0%	29.6%	9.4%	NA	20.7%
TOTAL for Sample Type	*Salmonella* positive	29.8%	5.0%	21.6%	28.4%	4.7%	19.9%	33.7%	19.9%
	Salmonella & *Listeria* positive	20.8%	1.1%	2.2%	1.6%	3.1%	0.9%	2.5%	4.0%
	Listeria positive	57.2%	25.7%	12.3%	3.2%	20.3%	9.5%	15.2%	19.4%

NA: none analyzed.

TABLE 3. Number of samples needed for analysis to provide a 95% confidence level of finding a positive result.

Plant Area		Sample Type: Drains	Walls, Floors, Fixtures	Equipment, Product Contact Surfaces	Process or Chilling Water	Personnel, Gloves, Boots, Aprons	Raw Product: Carcass, Parts, Ground	Fecal Contamination	TOTAL for Plant Area
Transport, Scalding, Defeathering	*Salmonella* positive	**6**	17	**7**	ND	0	ND	24	**8**
	Salmonella & *Listeria* positive	8	84	87	ND	0	ND	0	42
	Listeria positive	5	17	24	ND	0	ND	8	15
Evisceration, Gizzard Harvest, Reprocessing	*Salmonella* positive	**7**	106	17	0	0	39	**8**	14
	Salmonella & *Listeria* positive	9	0	111	0	0	0	106	42
	Listeria positive	6	15	30	0	18	21	24	14
Post-evisceration through Water Chilling	*Salmonella* positive	22	0	39	**10**	36	**9**	ND	19
	Salmonella & *Listeria* positive	45	0	348	168	36	0	ND	165
	Listeria positive	6	8	22	84	24	41	ND	18
Post-chill Cut-up, Grind, Packaging	*Salmonella* positive	60	216	36	12	81	16	ND	27
	Salmonella & *Listeria* positive	120	216	249	0	0	238	ND	235
	Listeria positive	5	10	23	0	10	32	ND	14
TOTAL for Sample Type	*Salmonella* positive	**10**	60	14	11	64	15	**9**	15
	Salmonella & *Listeria* positive	14	261	136	186	96	331	118	76
	Listeria positive	5	12	24	93	15	32	20	15

ND: not determined. 0: no samples tested positive

TABLE 4. *Salmonella* results by season.

Season Collected	*Salmonella* Positive	Samples Tested	*Salmonella* % positive
Spring	88	473	18.6
Summer	73	269	27.1
Fall	112	464	24.1
Winter	11	223	4.9
Total	284	1429	19.9