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Food Safety and Inspection Service

Office of Public Health Science

Microbiology Division

The Nationwide Microbiological Baseline Data Collection Program: Young Turkey Survey

August 2008 – July 2009

FOREWORD

This report provides an overview of the Nationwide Microbiological Baseline Data Collection Program: Young Turkey Survey and discusses the microbiological data results derived from young turkeys sampled during the twelve month time frame of August 2008 - July 2009. The program was designed and performed by the Food Safety and Inspection Service (FSIS) to estimate the percent positive and level of microbiological pathogens and indicator bacteria on raw turkey carcasses. The design and implementation of this survey was the result of the contribution of many offices and staff members from FSIS in the United States Department of Agriculture. The Microbiological Analysis and Data Branch, Division of Microbiology, Office of Public Health Science conducted this survey and prepared this report. The collection of samples was the responsibility of inspection personnel in the FSIS Office of Field Operations (OFO). The microbiological analyses for this survey were conducted by Food Safety Net Services, Ltd., San Antonio, TX.

NATIONWIDE MICROBIOLOGICAL BASELINE DATA COLLECTION PROGRAM: YOUNG TURKEY SURVEY

AUGUST 2008 – JULY 2009

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YOUNG TURKEY SURVEY

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EXECUTIVE SUMMARY

The Nationwide Microbiological Baseline Data Collection Program: Young Turkey Survey was performed to determine the presence and the concentration of selected bacteria on young turkey carcasses produced in federally inspected plants and to determine the National Prevalence Estimate for *Salmonella* and *Campylobacter* for this commodity. Two secondary goals of this survey were to determine if there was a significant difference between First Shift and Second Shift as it relates to bacterial presence and levels on turkey carcasses and to determine the level of reduction of bacteria between Re-Hang and Post-Chill. From August 2008 to July 2009, 2,884 swab samples from young turkey carcasses were collected from 58 establishments that slaughtered young turkeys and young breeder turkeys and produced whole carcasses under Federal inspection. Samples were taken at two different locations (Re-Hang and Post-Chill) in the production process and were collected from two separate shifts. These samples were analyzed to estimate the percent positive rate (percentage of samples for which the specific organism was found) and levels of *Salmonella*, *Campylobacter,* generic *Escherichia coli*, Aerobic Plate Count (APC), *Enterobacteriaceae*, and total coliforms. The prevalence for *Salmonella* and *Campylobacter* at Post-Chill was estimated from these data, and used to determine performance standards. The presence and concentration of the pathogens and indicator organisms were compared to determine if significant differences existed between samples taken at Re-Hang and Post-Chill locations between the separate shifts. The percent positive rate for the organisms from samples taken at Post-Chill was 1.66% for *Salmonella*, 88.0% for Aerobic Plate Count, 36.3% for *Enterobacteriaceae*, 30.0% for total coliforms, and 20.7% for generic *E. coli*. The percent positive rate for *Campylobacter*, calculated by combining qualitative and quantitative test results, was 1.46%. The estimated prevalence for *Salmonella* was 1.73% and for *Campylobacter*, 1.09%. From Re-Hang to Post-Chill there was a reduction in the percentage positive rate for both *Campylobacter* (Re-Hang – 22.68%, Post-Chill – 1.11%) and *Salmonella* (Re-Hang – 4.99%, Post-Chill – 0.35%). There was a statistical difference (p-value 0.05) for presence of generic *E. coli* at Re-Hang at Shift 1 when compared to Shift 2 (presence of generic *E. coli* was significantly higher at Shift 2) while the differences between shifts for *Salmonella* and *Campylobacter* were not statistically significant. With Post-Chill samples, the differences for generic *E. coli* between shifts were not statistically significant, while the number of samples positive for *Salmonella* and *Campylobacter* were so few that a valid comparison could not be made. The *Salmonella* serotypes isolated most often from the young turkey carcasses sampled at Re-Hang were Hadar (58 isolates), Schwarzengrund (15 isolates), and Saint Paul (12 isolates). From Post-Chill samples the serotypes most isolated were Hadar (13 isolates), Albany (2 isolates), and Heidelberg (2 isolates). Three of these serotypes, Heidelberg, Saint Paul, and Hadar, are among the top 20 most reported *Salmonella* serotypes isolated from human sources (4^{th}, 11^{th}, and 20^{th}, respectively).

INTRODUCTION

The Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) is responsible for the enforcement of the Federal Meat Inspection Act, the Poultry Products Inspection Act and the Egg Products Inspection Act. These Acts empower the Agency to inspect raw and processed meat, poultry, and egg products for evidence of insanitary conditions and adulteration. In addition, using provisions cited under these Acts, the Secretary of Agriculture is authorized to promote special assessments (such as baseline surveys) to estimate the presence (qualitative) and concentration (quantitative levels) of pathogens and indicator bacteria in raw products. Baseline surveys are statistically designed to assess the industry as a whole by weighting sampling of each establishment according to their relative production volume. Because the data are weighted by production volume, quantitative pathogen data from this and other baseline studies provide a scientific basis for exposure assessment modeling. This is a critical component of risk assessment; as is establishing microbiological

criteria or standards; assessing poultry production parameters; assessing the seasonal and regional variability in prevalence; and assessing levels of pathogen and indicator bacteria. Data collected during baseline studies are essential for meeting policy development and public health goals.

Previously, FSIS performed baseline surveys on young turkeys in 1996 and 1997. In an effort to enhance the quality of these surveys during this baseline, the Agency had a 90-day training period for personnel in the field and laboratory and created mailboxes where the inspection program personnel could submit questions about the survey. Formal FSIS Notices and training DVDs were used to provide the inspection program personnel information about the survey and instructions for sampling. Information gained during this "shake down" period was used to improve the actual survey.

Additionally, FSIS implemented several technical modifications during this baseline survey. These changes included:

- Sampling turkey carcasses at two points during processing: Re-Hang and Post-Chill. **Re-Hang** refers to the location in the process after the picker and removal of feet, prior to evisceration of the bird. **Post-Chill** refers to the point in the process where the turkeys exit the chiller after all slaughter interventions have taken place, but before entering coolers or proceeding to further processing.

- In establishments that reported having two production shifts, the sampling was conducted during the specified shift (Shift 1 or Shift 2). In establishments that reported a single production shift, all sampling events were documented as Shift 1[1].

- Based on the recommendation of the National Advisory Committee on Microbiological Criteria for Foods (NACMCF)[2], a *Campylobacter* analytical method was developed and used to analyze the samples for this bacterial pathogen. The NACMCF recommended method provided an expedient, high through-put, robust method for identifying and quantifying *Campylobacter.* The FSIS Microbiology Laboratory Guidebook (MLG) method Chapter 6. Isolation, Identification, and Enumeration of *Campylobacter jejuni/coli* from meat and poultry products was not appropriate for this survey because it was not suitable for meeting technical or logistic goals.

OBJECTIVES

This baseline survey had four primary objectives:

1. To collect microbiological data from young turkey swab samples in order to determine the presence and concentration of specific microbiological targets as an anchor point to measure change over time. Microbiological targets included:

   Pathogens:

   - *Salmonella*
   - *Campylobacter*

   Indicator bacteria:

[1] Generally, Shift 1 is defined as the time period of production that occurred immediately after a pre-operational sanitation inspection was performed, but this did not apply to all establishments in this baseline since each establishment is responsible for defining what a shift is within their plant. The shift information is entered into the FSIS Electronic Animal Disposition Reporting System (eADRS).

[2] This recommendation can be found within the NACMCF report, Analytical Utilities of *Campylobacter* Methodologies, on the FSIS web site at http://www.fsis.usda.gov/PDF/NACMCF_Campylobacter_092805.pdf.

- Generic *Escherichia coli (E. coli)*
- Total Coliforms
- Aerobic Plate Count (mesophilic)
- *Enterobacteriaceae*

2. To assess the effect of the slaughter process on microbiological contamination by comparing the prevalence and concentration of the selected bacteria between young turkey carcasses at Re-Hang and Post-Chill.

3. To provide data for use in the development of risk assessments, risk-based sampling programs, and/or regulatory policy decisions (including the development of future performance criteria and guidance).

4. To provide isolates of *Salmonella* and *Campylobacter* to research partners in order to generate subtyping and antimicrobial resistance data.

PROGRAM DESIGN

Establishments Included in the Sampling Frame

Eighty–two establishments identified for Federal inspection in the FSIS Electronic Animal Disposition Reporting System (eADRS) that slaughtered young turkeys and young breeder turkeys during the twelve-month period from April 2007 through March 2008 were included in the sampling frame and eligible for selection during this baseline survey.

These establishments contributed approximately 97% of the total number of young turkeys slaughtered in the U.S. under Federal Inspection during this period of time. In order to assess the effect of establishment size on the microbiological profiles of young turkey carcasses it was necessary to include low-volume establishments in this survey.

However, several small turkey slaughter establishments were not included in the final sampling frame because the products produced in these plants were not produced under federal inspection. Other establishments were removed from the sampling frame due to inspection withdrawal, suspension, or that the establishment had gone out of business prior to the start of the study. The final sampling frame included 72 establishments; however, several plants did not respond to the survey, reducing the number of establishments sampled to 58.

Sample Design

Factors considered in the design of this sampling program included the size and variability of the young turkey population, the nature and number of bacterial targets to be investigated, the practicality and limitations of sampling, the specific data to be collected, sampling costs, and the methods available for sampling and testing.

Two types of errors were considered. Those attributable to sampling and measurement and those related to operational deficiencies such as samples that were not analyzed due to unsatisfactory temperature constraints, or product not being produced when a request for sample was made. Sampling errors occur because observations are derived from a portion rather than from the entire population; non-sampling errors may be attributed to many sources inherent to the collection of samples, laboratory analysis and data analysis.

The Nationwide Microbiological Baseline Data Collection Program: Young Turkey Survey follows a stratified design where an establishment is first selected within a stratum and then samples within an establishment are chosen. Establishments were assigned to one of five production volume categories.

The number of samples per establishment depended upon the volume category. The production shift during which a sample was collected was specified.

- **Sampling Event Frequency Category 1** consisted of establishments that slaughtered ≥6,000,000 young turkeys during the year. The frequency for sampling events (single samples collected from both Re-Hang and Post-Chill locations) in these establishments was five times per month (i.e., 60 sampling events in an establishment per year).

- **Sampling Event Frequency Category 2** consisted of establishments that slaughtered ≥ 1,500,000 but < 6,000,000 young turkeys during the year. The sampling frequency for establishments in this category was three times per month (i.e., 36 sampling events in an establishment per year).

- **Sampling Event Frequency Category 3** consisted of establishments that slaughtered ≥ 10,000 but < 1,500,000 young turkeys AND slaughtered young turkeys for ≥ 10 months during the year. The sampling frequency for establishments in this category was two times every month (24 sampling events in an establishment per year).

- **Sampling Event Frequency Category 4** consisted of establishments that slaughtered ≥ 10,000 but < 1,500,000 young turkeys AND slaughtered young turkeys for ≤ 9 months during the year. The sampling frequency for establishments in this category was four times each month during the period from September through December only (16 sampling events in an establishment per year).

- **Sampling Event Frequency Category 5** consisted of establishments that slaughtered < 10,000 young turkeys during the year. The sampling frequency for establishments in this category was two times each month during the period from September through December only (8 sampling events in an establishment per year).

Note about Categories 4 and 5: Sampling events in establishments that fell in these two categories were restricted to the period from September through December. The eADRS data showed that some establishments were inconsistent in their monthly slaughter totals across the year but had known seasonal slaughter cycles. In addition, for low volume establishments, the slaughter totals found in eADRS were the highest during the months of September through December or the totals during these months represented 100% of the annual total. Sampling during September through December provided the best approach that both facilitated the inclusion of low-volume establishments in the survey and provided the possibility of sampling the majority of the young turkey carcasses produced by these establishments.

After randomly assigning the shift (Shift 1 or Shift 2) for collection of the first sample in an establishment, subsequent sample requests alternated between shifts. In establishments that reported a single production shift, all sampling forms indicated that sampling would occur on Shift 1. For the purposes of this survey, the shift was defined to be consistent with data entry for shift slaughter totals in eADRS.

Sampling Location within the Establishment

To evaluate the cumulative effects of sanitary dressing and slaughter interventions, carcasses were swabbed at **Re-Hang** and **Post-Chill** locations. Swabs were collected throughout the year from carcasses at both Re-Hang and Post-Chill locations and from multiple production shifts in establishments, except as noted above for Category 4 and 5 plants.

Sample Collection and Description

Samples were aseptically collected by FSIS inspection program personnel following the procedures in FSIS Directive 10,230.5 (2/4/98), the DVD entitled “Sampling Raw Meat and Poultry for *Salmonella*”, the

instructions provided on computer-generated sample collection request forms, and the specific instructions applicable to this program. For each sampling event, one randomly selected Re-Hang turkey carcass and one Post-Chill turkey carcass from the same grow-out flock/house was aseptically swabbed using sponges moistened with 10-25 ml pre-chilled Buffered Peptone Water (BPW). Because *Salmonella* and *Campylobacter* analyses could not be performed on the same swab sample, two swab samples were taken, one each for the right and left sides of the same carcass. For each carcass side, 50 cm^2 of the back and another 50cm^2 of the thigh were sampled using the same swab. In this manner, the consistency of sampling was maintained for *Salmonella*, *Campylobacter* and indicator organism testing. Once the carcass was swabbed, the swabs were placed in corresponding Whirl-Pak® bags with the remaining BPW solution, and put into individual resealable bags. These bags were placed in an insulated shipping container with gel packs capable of maintaining the proper temperature, and shipped to the laboratory by an overnight delivery service on the same calendar day they were collected. The samples were collected Monday through Friday during slaughter operations. Only those samples received at the laboratory the day after sample collection, with a sample receipt temperature of 0°C to 15°C (inclusive) were analyzed. Samples received outside this temperature range were not analyzed.

SELECTION OF ORGANISMS

Two pathogenic microorganisms were selected for analysis: *Salmonella* and *Campylobacter*. In addition, several organisms were selected as microbial indicators of sanitation and process control on young turkey carcasses: generic *E. coli*, mesophilic Aerobic Plate Count (APC), *Enterobacteriaceae*, and total coliforms.

ANALYTICAL METHODS

Indicator Bacteria

To analyze the swab samples for the indicator bacteria, 1 ml of Buffered Peptone Water (BPW) from the sample was added to 9.0 ml of a BPW diluent blank (10^{-1} dilution) and vortexed. In addition, serial dilutions from 10^{-2} to 10^{-4} were made and, after mixing, the dilutions were plated onto the appropriate Petrifilm™, in duplicate, to enumerate *Enterobacteriaceae* (1), generic *E. coli* (2), total coliforms (2), and to perform the APC (3). Results were reported in CFU/square centimeter (CFU/cm^2). The limit of detection (LOD) for these methods is 1.20 CFU/cm^2 based on swab diluent volume of 25 ml and the area swabbed of 100 cm^2 (50 cm^2 on each of the left and right sides of the carcass).

Salmonella

To provide the opportunity for follow-up quantitative testing for *Salmonella*, approximately 4 ml of the BPW was removed and stored at 4°C. The remaining volume of BPW (after indicator portions were removed) and the swab itself were analyzed with a qualitative method for *Salmonella* by adding 50 ml of pre-chilled BPW, massaging the swab by hand to ensure distribution of the BPW, and incubating the enrichment overnight at 35°C. Following incubation, an aliquot of the homogenate was screened for *Salmonella* using the DuPont BAX® PCR system (4) (5). The reserve BPW from the presumptively positive samples was tested for concentration of *Salmonella* using the "Most Probable Number" (MPN) estimation method (6). In a three-tube MPN, serial dilutions were made to represent 1.0 ml, 0.1 ml, and 0.01 ml from the swab homogenate. The pattern of positive and negative results among these individual qualitative tests was used to statistically estimate low levels of *Salmonella* and the results were calculated and reported as MPN/cm^2. The presence of *Salmonella* in all positive tubes was confirmed culturally. Those *Salmonella* MPN results where at least one tube was positive for *Salmonella* were labeled as "quantifiable" samples in the data tables of this report. The LOD for the MPN procedure is 0.3 MPN/ml or 0.075 MPN/cm^2, again based on the BPW test portion volume, carcass area swabbed and the appropriate MPN tables (6).

Campylobacter

To detect and enumerate *Campylobacter*, the swab samples were analyzed using two separate methods. A Quantitative Detection and Enumeration method [7], which was derived from a recommendation from NACMCF, and developed by USDA/Agricultural Research Service, was used on Post-Chill and Re-Hang carcass swab samples. The Qualitative Detection method, which was used only with the swab obtained from Post-Chill samples, included an enrichment step.

1. Qualitative Detection

For this analysis, 25 ml of Blood Free 2X Bolton's Enrichment Broth was added to the swab samples and incubated for 48 hours to allow as few as one cell of *Campylobacter* to multiply to levels that could be detected by screening and agar plating procedures. After incubation, a portion of this culture was inoculated onto Campy-Cefex plates. The plates were incubated at 42 ± 1.0°C for 48 ± 2 hours using a tri-gas incubator or equivalent, flushed to 85% nitrogen, 10% carbon dioxide and 5 % oxygen. Colonies that exhibited the characteristic colonial morphology of *Campylobacter* were confirmed as *Campylobacter* species (*C. coli*, *C. jejuni* or *C. lari*) by latex agglutination, and those samples identified as positive. Plates on which there were no *Campylobacter* colonies were identified as negative.

2. Quantitative Detection and Enumeration

Aliquots of BPW from sponges collected at Post-Chill and Re-Hang were plated directly onto Campy-Cefex agar plates. A 250 µl aliquot was plated directly on each of four Campy-Cefex plates for a total of 1ml examined. A ten-fold dilution of the swab was obtained by plating 100 µl directly on each of two Campy-Cefex plates and a mean count determined. If necessary, the BPW from these samples would be further diluted with sterile BPW and 0.1 ml of the dilution plated directly onto Campy-Cefex plates. The plates were incubated at 42 ± 1.0°C for 48 ± 2 hours using a tri-gas incubator or equivalent, flushed to 85% nitrogen, 10% carbon dioxide and 5 % oxygen. After incubation, colonies that exhibited the characteristic *Campylobacter* morphology were counted and up to 5 colonies of each morphology (if there was more than one) was speciated (*C. coli*, *C. jejuni* or *C. lari*) by latex agglutination. These samples were identified as positive and the bacterial counts recorded as colony forming units (CFU) per centimeters squared (cm^2) of turkey carcass swab. Plates on which there were no *Campylobacter* colonies were identified as negative. The LOD for this method is 0.25 CFU/cm^2 based on the area swabbed.

3. Sequence of analysis, Post-Chill samples

For the analysis of the Post-Chill samples, both media for the Quantitative Detection and Enumeration method and the Qualitative Detection method were inoculated at the same time. If colonies were detected on the Campy-Cefex plates for the Quantitative Detection and Enumeration method, the Qualitative Detection method was stopped. However, if there were no colonies detected on the Campy-Cefex plates for the Quantitative Detection and Enumeration method, the Qualitative Detection method was continued. If both methods were determined to be negative, the Post-chill sample was identified as negative for both tests.

4. Theoretical Limit of Detection

For the Quantitative Detection and Enumeration method, the maximum amount of the undiluted carcass swab analyzed was 1 ml, so the theoretical limit of detection for this assay is one colony per ml. Samples that were negative on this test were reported to be "<0.25 CFU/cm^2" in this report.

For the Qualitative Detection method, 25 ml of Blood Free 2X Bolton's Enrichment Broth was added to the carcass swab to allow *Campylobacter* to multiply to levels that could be detected by the agar plating and confirmation procedures. Because this method contains an enrichment step, the actual quantity of *Campylobacter* in the original carcass swab cannot be determined. However, the theoretical limit of detection for this assay is one cell per 100 cm^2 of carcass surface, or 0.01 cfu/cm^2. Samples which were negative for this test were reported to be "<0.25 CFU/cm^2" in this report.

RESULTS

A total of 2,884 samples from 58 establishments were collected and analyzed from young turkey carcasses during this survey. Because only paired samples were processed in the laboratory, there were an equal number (1,442 each) of Re-Hang and Post-Chill samples analyzed.

Table 1 presents a summary of the test results of samples that were analyzed and combines the results from both shifts. In addition, the data have been shown for both Re-Hang and Post-Chill. For indicator organisms, the number of samples quantified, number of positive samples and percent positive were provided. The arithmetic mean, mean standard error, the geometric mean (with a 95% confidence interval) and the $\log_{10}$ of the geometric mean are provided. Of note, for *Campylobacter*, only the results from the quantitative detection and enumeration method are presented in this table. At the bottom of the Table 1, an estimation of the percent positive and a 95% confidence interval is given for the pathogenic organisms.

For Re-Hang samples, 99.7% of the samples had detectable APC while 96.7% of the samples were above the LOD for *Enterobacteriaceae* microorganisms, and 95.8% and 92.2% of the samples were positive for total coliforms and generic *E. coli*, respectively (Table 1).

For Post-Chill samples, the percent positive rates were significantly lower ($p<0.05$) than their Re-Hang counterparts. The percent positive rates for APC, *Enterobacteriaceae,* total coliforms and generic *E. coli* were respectively, 88.0%, 36.3%, 30.0% and 20.7% (Table 1).

Comparisons between the means of presence of the organism at Re-Hang and at Post-Chill were made (Table 1). The results show that all levels of all the bacterial targets are significantly lower at Post-Chill when compared to the Re-Hang.

When the percent positive rates of *Salmonella* were compared between Re-Hang and Post-Chill samples, the percent positive rates were 4.99% and 0.35%, respectively. When comparing the Re-Hang and Post-Chill samples for *Campylobacter* the percent positive rates were 22.68% and 1.11%, respectively. The percent positive rates should not be considered as the national prevalence for these pathogens but rather the percent positive sample results observed during this survey.

Table 2 indicates that 24 Post-Chill, samples were found with *Salmonella* from 1,442 (1.66% positive) analyzed samples. The Post-Chill percent positive rate for the qualitative (0.35%) and for the quantitative (1.11%) *Campylobacter* test results were combined yielding a 1.46% positive rate. While the NACMCF recommendations for *Campylobacter* analysis specified a quantitative method only (direct plate counts on solid media), it was suspected that the levels of *Campylobacter* on turkey carcasses at Post-Chill may be too low to be detected using this method. During the shake down period, preliminary analysis of turkey rinse samples using only direct plating confirmed this theory and it was determined that a qualitative method should be added. During the actual survey, a portion of the swab homogenate was qualitatively analyzed by an enrichment and detection method for the Post-Chill samples only. However, because there was an enrichment step in the procedure, only qualitative results (positive or negative) were obtained from these samples.

The National Prevalence was calculated using a model-based volume-weighted estimate of prevalence that accounted for establishment/month variation. The prevalence estimate at Post-Chill for *Salmonella* was 1.73%, with a standard error of 0.307%. For *Campylobacter* the prevalence estimate at Post Chill was 1.09%, with a standard error of 0.268%. Details of the derivation of these estimates can be obtained at http://www.fsis.usda.gov/PDF/Technical_Paper_Performance_Guidance_Broilers.pdf.

For Re-Hang, the APC (35°C) were distributed such that 51.4% of the samples contained between 1,200.1 and 12,000.0 microorganisms while for Post-Chill samples the APC (35°C) were distributed such that 45.2% of the samples were between 1.2 and 12.0 microorganisms.

For generic *E. coli* positive samples, the highest percentage of Re-Hang samples contained between 12.1 and 120.0 cfu. (Table 3) For Post-Chill samples, the greatest percentage fell between the distribution level of 1.2 and 12.0 CFU (Table 6).

Of the 1,442 Re-Hang samples tested for *Campylobacter*, 327 were confirmed positive for *Campylobacter* by *q*uantitative analysis. Of the quantifiable samples, 157 (48.01%) had a quantitative range from 0.25 to 2.50 CFU/cm^2 and 134 (48.01%) of the samples ranged from 2.51 - 25.0 CFU/cm^2. The remaining ranges for *Campylobacter* are reported in Table 4, with one sample within the highest range, 250.1 - 2500 CFU/cm^2 (Table 4).

Only 16 Post-Chill samples confirmed as *Campylobacter*-positive and, as expected, the levels of *Campylobacter* in these samples were much lower. Of the 1,442 Post-Chill samples tested for *Campylobacter*, 1,426 were below the LOD. Of the 16 positive samples, 13 (81.25%) had a quantitative range from 0.25-2.5 CFU/cm^2, 2 (12.50%) had a quantitative range from 2.51-25.0 CFU/cm^2, and 1 sample (6.25%) within the 25.1 to 250.0 CFU/cm^2 range (Table 7).

Of the Re-Hang *Salmonella* samples, 72 were confirmed with 1370 samples below the LOD. Of the positive samples, 54 (75.00%) ranged from 0.075 to 0.75 MPN/cm^2 and 11 (15.28%) samples ranged from 0.751 to 7.50 MPN/cm^2. Six samples were within the range of 7.51-75.0 MPN/cm^2 with 1 sample undetermined (Table 5).

As expected, there were many fewer Post-Chill samples that confirmed *Salmonella*-positive. Of the 1,442 samples tested, 4 (80%) had a quantitative range from 0.075-0.75 MPN/cm^2, and 1 sample (20%) ranged from 0.75-17.5 MPN/cm^2 (Table 8).

The *Salmonella* serotypes isolated most often at Re-Hang were Hadar (58), Schwarzengrund (15), and Saintpaul (12). From Post-Chill samples the serotypes most isolated were Hadar (13), Albany (2), and Heidelberg (2).

Tables 9 and 10 present the results from samples collected at Re-Hang and Post-Chill from plants that had two production shifts. For the purpose of identifying differences, a comparison of the average concentration of the organisms at Shift 1 Re-Hang and at Shift 2 Re-Hang was performed. This same comparison of average concentration was done for the Shift 1 Post-Chill and Shift 2 Post-Chill samples. Given that the distribution of pathogens are not normally distributed, a non-parametric test Wilcoxon/Kruskal-Wallis (rank sums) was performed. The statistical tests (p-value < 0.05) showed a significant difference for Generic *E. coli* at Re-Hang during Shift 1 when compared to Shift 2 (presence of Generic *E. coli* was significantly higher at Shift 2) while there was no significant differences between shifts for *Salmonella* and *Campylobacte*r. With Post-Chill samples, the differences for Generic *E. coli* between shifts were not significantly different, while the number of samples positive for *Salmonella* and *Campylobacter* were too few for a valid comparison.

DISCUSSION

The Nationwide Microbiological Baseline Data Collection Program: Young Turkey Survey was designed to determine the presence and the concentration of selected bacteria on young turkey carcasses produced in federally inspected plants. In 1997 FSIS conducted a similar baseline survey for *Salmonella* and generic *E. coli* (Nationwide Sponge Microbiological Baseline Data Collection Program: Young Turkeys [8]). This survey, unlike the previous survey, included testing for *Campylobacter*, *Enterobacteriaceae*, Aerobic Plate Count (mesophilic) and Total Coliforms. Technical modifications such as sampling at two process locations and during two production shifts were made, as well as using a new *Campylobacter* direct plating method.

In addition to obtaining the percent positive and levels of various bacteria in turkey sponge samples, there were a number of additional goals for this survey. One goal was to determine if there was a significant difference between First and Second Shift as it relates to bacterial levels on turkey carcasses. It was expected that bacterial levels on turkey carcasses would be lower during the first shift, but, as turkey slaughter continued during the day, the bacterial concentration levels would increase.

Our analysis indicated that there was a statistically significant difference in the levels of Generic *E. coli* analyzed between First and Second shift, suggesting that at least in these plants, the length of time from clean-up to sample collection does influence the levels of these bacteria on turkey carcasses. However, the presence of *Salmonella* and *Campylobacter* were not detected at a significantly higher incidence at Re-Hang Second Shift vs. First Shift. This finding most likely is not important since at Post-Chill, which would be considered closer to Retail and thus, the consumer, the presence of both bacterial pathogens was determined to be extremely low.

A second goal of this survey was to determine the level of reduction of bacteria between Re-Hang and Post-Chill. A substantial reduction would be expected because of the various anti-microbial interventions that would occur prior to immersion in the chill tank or, in some plants, after the turkey carcasses are removed from the chill tank. In Table 1, we note a substantial reduction in the number of samples positive for *Salmonella* from Re-Hang to Post-Chill (4.99% and 0.35%) and *Campylobacter* (22.68% and 1.11%), suggesting that the anti-microbial interventions were effective. The prevalence estimate for *Salmonella* was 1.73%, with a standard error of 0.307%. For *Campylobacter* the prevalence estimate was 1.09%, with a standard error of 0.268%. Details of the derivation of these estimates can be obtained at http://www.fsis.usda.gov/PDF/Technical_Paper_Performance_Guidance_Broilers.pdf.
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Among the top three *Salmonella* strains we identified, via serotyping, three of them were of human public health concern. These strains, Heidelberg, Saint Paul, and Hadar, have been reported by the Centers for Disease Control, to be in the top 20 *Salmonella* serotypes from human sources (4^{th}, 11^{th}, and 20^{th}, respectively) [(9)]. This indicates that young turkeys habor *Salmonella* strains that have the potential to cause human illness.

During this survey, a new method for the analysis of *Campylobacter* in samples obtained from turkey carcasses was implemented. The analytical method, recommended by the NACMCF, is a direct plating method that enables the direct enumeration of *Campylobacter* from turkey swab homogenates, thus giving an indication of the actual level of *Campylobacter* contamination of the carcass. In the majority of samples analyzed, the *Campylobacter* concentration on Post-Chill carcasses was too low to detect by direct plating. This suggests that the process control(s) used in these plants are effective at reducing the concentration of these bacteria to very low levels. A qualitative analytical procedure that enriched specifically for this pathogen and would allow for the detection of lower levels of this bacterium was then added. The addition of this method made it possible to detect more carcasses that were actually positive for *Campylobacter* but would not allow us to quantify these bacteria. The agency is in the process of adopting and implementing the *Campylobacter* method used in this baseline as an official analytical method in the FSIS Microbiological Laboratory Guidebook.

TABLES

Table 1. Comparison between Re-Hang and Post-Chill Samples by Microorganism in the 2008 – 2009 Young Turkey Survey

Microorganisms					Levels of Positives				
Indicator Organism	Sample Collected at	Number of Samples Tested	Number of Samples Quantifiable (1)	Percent Positive	Mean (CFU/cm^2) (2)	Mean Std Error	Geometric Mean	Geo Mean 95% CI	Log 10 of the Geo Mean
Aerobic Plate Count	Re-Hang	1,442	1,438	99.7%	105,784.0	85,087.0	1,623	(1492 - 1767)	3.21
	Post-Chill	1,442	1,269	88.0%	819.0	216.0	25	(22.5 - 28. 0)	1.40
Enterobacteriaceae	Re-Hang	1,442	1,394	96.7%	412.0	79.0	56.1	(51.7 - 61.7)	1.75
	Post-Chill	1,442	523	36.3%	178.8	162.5	5	(4.4 - 5.4)	0.69
Total Coliforms	Re-Hang	1,442	1,382	95.8%	347.0	69.0	44	(40.2 - 48.2)	1.64
	Post-Chill	1,442	433	30.0%	11.2	2.3	4	(3.9 - 4.7)	0.63
Generic *Escherichia coli*	Re-Hang	1,442	1,330	92.2%	202.8	40.0	29	(26.5 - 31.6)	1.46
	Post-Chill	1,442	299	20.7%	10.2	2.7	3.9	(3.56 - 4.35)	0.59
Pathogenic Organism									
Campylobacter	Re-Hang	1,442	327	22.68%	11.86	1.74	3.4	(2.89 - 4.01)	0.53
	Post-Chill	1,442	16	1.11%	3.4	1.9	0.9	(0.40 - 2.03)	-0.04
Salmonella (3)	Re-Hang	1,442	72	4.99%	3.08	0.86	0.45	(0.29 - 0.68)	-0.34
	Post-Chill	1,442	5	0.35%	1.02	0.69	0.50	(0.11 - 2.24)	-0.30

(1) Limit of Detection (LOD) for Aerobic Plate Count, *Enterobacteriaceae*, Total Coliforms and Generic *E. coli* = 1.2 CFU/cm^2; LOD for *Salmonella* = 0.075 MPN/cm^2; LOD for *Campylobacter* = 1 CFU/ml

(2) All mean differences between Re-Hang and Post-Chill are statistically significantly different.

(3) *Salmonella* measurements are in MPN/cm^2

Table 2. Post-Chill Summary of Percent Positives for *Campylobacter* and *Salmonella* in the Young Turkey Survey

Pathogenic Organism	Test type	Number of Samples	Number of Positives	Percent Positive
Campylobacter	**Quantitative Method [(1)]**	**1,442**	**16**	**1.11%**
	Qualitative Method [(2)]	**1,426**	**5**	**0.35%**
	Total combined [(3)]	**1,442**	**21**	**1.46%**
Salmonella	**Qualitative Method [(4)]**	**1,442**	**24**	**1.66%**

(1) Refer to Table 1.

(2) Represents data for qualitative enrichment-based testing. This test was conducted for all Post-Chill samples that were not positive by the quantitative test.

(3) Because qualitative testing was conducted only on those samples that were not positive for *Campylobacter* by the quantitative test, these data are combined for the total percent positive.

(4) For *Salmonella*, all samples were tested using the qualitative method. A follow up MPN method was done on qualitative-positive samples and then converted to CFU/cm^2 (See results in Table 1).

Table 3. Distribution of Quantified Generic *Escherichia coli* - Re-Hang Samples

Range, CFU/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
1.2 - 12.0	457	34.36	457	34.4
12.1 - 120.0	642	48.27	1,099	82.6
120.1 - 1,200.0	206	15.49	1,305	98.1
1200.1 - 12,000.0	20	1.50	1,325	99.6
12,000.1 - 120,000.0	5	0.38	1,330	100.0
Total	1,330	100.00		

Table 4. Distribution of Quantified *Campylobacter* - Re-Hang Samples

Range, CFU/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
0.25 - 2.50	157	48.01	157	48.0
2.51 - 25.0	134	40.98	291	89.0
25.1 - 250.0	35	10.70	326	99.7
250.1 - 2,500.0	1	0.31	327	100.0
Total	327	100.00		

Table 5. Distribution of Quantified *Salmonella* - Re-Hang Samples

Range, MPN/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
0.075 - 0.750	54	75.00	54	75.0
0.751 - 7.50	11	15.28	65	90.3
7.51 - 75.0	6	8.33	71	98.6
Undetermined	1	1.39	72	100.0
Total	72	100.00		

Table 6. Distribution of Quantified Generic *Escherichia coli* - Post-Chill Samples

Range, CFU/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
1.2 - 12.0	273	91.30	273	91.3
12.1 - 120.0	23	7.69	296	99.0
120.1 - 1,200.0	3	1.00	299	100.0
Total	299	100.00		

Table 7. Distribution of Quantified *Campylobacter* - Post-Chill Samples

Range, CFU/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
0.25 - 2.50	13	81.25	13	81.3
2.51 - 25.0	2	12.50	15	93.8
25.1 - 250.0	1	6.25	16	100.0
Total	16	100.00		

Table 8. Distribution of Quantified *Salmonella* - Post-Chill Samples

Range, MPN/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
0.075 - 0.750	4	80.00	4	80.0
0.751 - 7.50	1	20.00	5	100.0
Total	5	100.0		

Table 9. Statistical Comparison between Re-Hang Shift 1 and Shift 2 Samples in the 2008 – 2009 Young Turkey Survey [1]

Microorganism	Shift 1					Shift 2					P-value [3]
	Sample Results	Mean [2]	Std Dev	Geo Mean	log 10 of Geo Mean	Sample Results	Mean [2]	Std Dev	Geo Mean	log 10 of Geo Mean	
Generic *E. coli* (CFU/cm^2)	361	195	1,204	36.2	1.55	338	234	1,587	29.5	1.47	0.04 [4]
Campylobacter (CFU/cm^2)	79	7.0	11.5	2.9	0.46	65	15.4	26.0	4.79	0.68	0.10 [5]
Salmonella (MPN/cm^2)	25	1.8	5.9	0.31	-0.50	23	2.5	6.3	0.36	-0.44	0.66 [5]

(1) Results for plants with only one shift are not included in this comparison.
(2) For the purpose of identifying differences, a comparison of the average concentration of the organisms at Shift 1 Re-Hang and at Shift 2 Re-Hang was performed
(3) Distributions are not Normal. Non-Parametric Wilcoxon/Kruskal-Wallis Test (Rank Sums) was performed.
(4) There is statistical difference at Re-Hang at Shift 1 when compared to Shift 2.
(5) There is no statistical difference at Re-Hang between Shift 1 and Shift 2.

Table 10. Statistical Comparison between Post-Chill Shift 1 and Shift 2 Samples in the 2008 – 2009 Young Turkey Survey [1]

Microorganism	Shift 1					Shift 2					P-value ([3])
	Sample Results	Mean [2]	Std Dev	Geo Mean	log 10 of Geo Mean	Sample Results	Mean [2]	Std Dev	Geo Mean	log 10 of Geo Mean	
Generic *E. coli* (CFU/cm^2)	71	4.15	4.09	3.32	0.52	63	14.20	77.90	3.58	0.55	0.60 ([4])
Campylobacter (CFU/cm^2)	2	0.25	0.00	0.25	-0.60	5	0.30	0.11	0.28	-0.54	([5])
Salmonella (MPN/cm^2)	-	-	-	-	-	1	0.58	-	-	-	([5])

(1) Results for plants with only one shift are not included in this comparison.
(2) For the purpose of identifying differences, a comparison of the average concentration of the organisms at Shift 1 Post-Chill and at Shift 2 Post-Chill was performed
(3) Distributions are not Normal. Non-Parametric Wilcoxon/Kruskal-Wallis Test (Rank Sums) was performed.
(4) There is no statistical difference at Re-Hang between Shift 1 and Shift 2.
(5) Positive results of sample are too small for a valid test.

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