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Microbiology Division

The Nationwide Microbiological Baseline Data Collection Program: Young Chicken Survey

July 2007– June 2008

FOREWORD

This report provides an overview of the The Nationwide Microbiological Baseline Data Collection Program: Young Chicken Survey and discusses the microbiological data results derived from young chickens sampled during the twelve month time frame of July 2007 - June 2008. 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 chicken 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, Microbiology Division, 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.

THE NATIONWIDE MICROBIOLOGICAL BASELINE DATA COLLECTION PROGRAM: YOUNG CHICKEN SURVEY

JULY 2007 – JUNE 2008

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EXECUTIVE SUMMARY

From July 2007 to June 2008, 6,550 rinsate samples from young chicken carcasses were collected at 182 establishments that slaughtered young chickens and produced whole carcasses under Federal Inspection. Samples were taken at two different location points (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 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 specific microbiological targets were compared to determine if significant differences existed between samples taken at Re-Hang and Post-Chill and during the separate shifts. The percent positive rate for the organisms from samples taken at Post-Chill was 8.15% (qualitative) for *Salmonella*, 97.07% for the Aerobic Plate Count, 57.40% for *Enterobacteriaceae*, 47.82% for Total Coliforms, and 38.66% for Generic *Escherichia coli*. The percent positive rate, compositing qualitative and quantitative test results, for *Campylobacter* was 46.60%. The estimated prevalence for *Salmonella* was 7.5%, and for *Campylobacter,* 46.7%. When quantitative results from Re-Hang and Post-Chill were compared, a reduction in the percentage positive rate was observed for both *Campylobacter* (Re-Hang – 71.36%, Post-Chill – 10.66% at P-value < 0.05, p=0.00) and *Salmonella* (Re-Hang – 40.70%, Post-Chill – 5.19%, at P-value < 0.05, p=0.00). In comparing the levels of *Campylobacter*, *Salmonella* and Generic *Escherichia coli* between Shift 1 and Shift 2 (in plants that have two production shifts) there were no statistically significant differences (P>0.05) between the shifts when comparing the levels of each bacteria. The *Salmonella* serotypes isolated most often from the young chicken samples during this survey were Kentucky, Heidelberg, Typhimurium and Typhimurium (Copenhagen), and these findings were consistent regardless of shift or whether the sample was obtained at Re-Hang or Post-Chill.

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 studies, to estimate the presence (qualitative) and number (quantitative levels) of pathogens and indicator bacteria in raw products. Baseline studies 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 is weighted by production volume, quantitative pathogen data from this and other baseline studies provide a scientific basis for exposure assessment. This is a critical component of risk assessment, establishing microbiological criteria or standards, assessing poultry production parameters, and assessing the seasonal and regional variability in prevalence and levels of pathogen and indicator bacteria. Data collected during baseline studies is essential for meeting these mission-critical needs.

FSIS performed baseline studies on young chickens in 1999 and 1994. In efforts to continuously enhance the quality of these studies, during this baseline the Agency had a 90-day training period for the field and laboratory personnel, created mailboxes where OFO inspection program

personnel could submit questions about the survey, and used formal FSIS Notices and training DVDs to provide the inspection program personnel information about the survey and instructions for sampling.

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

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

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

3. 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 current FSIS Microbiology Laboratory Guidebook (MLG) method for *Campylobacter* was not appropriate for this survey because it was laborious; therefore, an expedient, high through-put, robust method for identifying and quantifying *Campylobacter* was needed.

OBJECTIVES

This baseline survey had four primary objectives [(1)]:

1. To collect microbiological data from young chicken rinsate 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:

   - Generic *Escherichia coli (E. coli)*
   - Total Aerobic Bacteria
   - *Enterobacteriaceae*
   - Coliforms

[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.

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

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

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

PROGRAM DESIGN

Establishments Included in the Sampling Frame

Federal establishments identified in the FSIS Electronic Animal Disposition Reporting System (eADRS) that slaughtered a minimum of 100,000 young chickens in fiscal year (FY) 2006 (i.e. the twelve-month period from October 1, 2005 through September 30, 2006) were included in the sampling frame and eligible for selection during this baseline survey.

The slaughter totals available in eADRS only specify young chicken production and do not differentiate among specific types of young chickens (broilers, roasters, Cornish hens, etc). FSIS had discontinued sub classification of broilers among "young chickens", but because broilers make up the majority of the young chickens the 2007 – 2008 survey design specified that only broilers be collected for this program.

There were approximately 200 establishments identified in eADRS as slaughtering young chickens in FY2006. These establishments contributed 99.994% of the total head of young chickens slaughtered in the U.S. under Federal Inspection during FY2006. Several of these establishments were not included in the sampling frame because the products were considered religious exempt and did not bear the mark of inspection. Other establishments were removed from the frame due to inspection withdrawal, because the plants did not produce the appropriate class of birds requested for sampling or because the plants did not fall within a production category. The final sampling frame included 182 establishments.

Sample Design

Many factors were considered in the design of this sampling program. Among these were the size and variability of the young chicken population, the nature and number of bacteria 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, sampling errors attributable to sample size and non-sampling errors, for example, due to laboratory methodology. Both sampling and non-sampling errors may affect the reliability of results and, thus, had to be considered in designing this program. 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 in the collection of samples, laboratory analysis and processing of data. These types of errors were considered in determining the total sample size and the specific number of samples to be collected from each establishment.

The Nationwide Young Chicken Microbiological Baseline Survey of Young Chicken Carcasses incorporated a multistage cluster design that included sampling in establishments over time. In all establishments included in the sampling frame, individual broiler carcasses were selected at

intervals defined according to each of three production volume categories. For establishments in certain categories, the production shift during which a sample was collected was specified.

Production Volume Category 1 consisted of establishments that slaughtered ≥ 90,000,000 head of young chickens in FY2006. Carcass rinses were collected two times per month (24 sampling events in an establishment per year) from establishments in this category.

Production Volume Category 2 consisted of establishments that slaughtered ≥ 25,000,000 but < 90,000,000 head of young chickens in FY2006. Carcass rinses were collected once per month (12 sampling events in an establishment per year) from establishments in this category.

Production Volume Category 3 consisted of establishments that slaughtered ≥ 100,000 but < 25,000,000 head of young chickens in FY2006. Carcass rinses were collected once every two months (6 sampling events in an establishment per year) from establishments in this category.

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 requests 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.

It was estimated that at least 4,500 carcass rinses would need to be collected during 2,250 sampling events[3] per year to ensure reasonable levels of precision based on the projected prevalence for the bacterial targets included in this baseline survey.

Sampling Location within the Establishment

To evaluate the cumulative effects of sanitary dressing and slaughter interventions, carcass rinses were collected and sampled at **Re-Hang** and **Post-Chill** locations. Rinsates were collected throughout the year from carcasses at both Re-Hang and Post-Chill locations and from multiple production shifts in establishments.

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*", instructions provided on computer-generated sample collection request forms, and specific instructions applicable to this program. For each sampling event, one randomly selected Re-Hang broiler carcass and one Post-Chill broiler carcass from the same grow-out flock/house was aseptically placed into its own sterile bag and shaken with 400 ml of pre-chilled Buffered Peptone Water (BPW). Once the contents of the bags were properly mixed, two sterile screw-cap containers with properly labeled lids were each filled with all of the rinse fluid. The two sample containers were sealed, put into individual resealable bags, placed in an insulated shipping container with gel packs capable of maintaining the proper temperature, and shipped to the contract 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^{o}C to 10^{o}C (inclusive) were analyzed. Samples received outside this temperature range were not analyzed.

[3] A sampling event consists of one Re-Hang rinsate and one Post-Chill rinsate being collected concurrently.

SELECTION OF ORGANISMS

In order to obtain microbiological data for use in the development of risk assessments, risk-based sampling programs and/or regulatory policy decisions, and to obtain up to date microbiological data for comparison to findings from earlier baseline studies (where appropriate), the samples were analyzed for a number of microorganisms. Two pathogenic microorganisms were selected for analysis: *Salmonella* and *Campylobacter*. In addition, several organisms were selected as microbial indicators of sanitation and general microbial presence on young chicken carcasses: generic *E. coli*, total aerobic bacteria, *Enterobacteriaceae*, and coliforms.

ANALYTICAL METHODS

Indicator Bacteria

To analyze the samples for the indicator bacteria, 1ml of rinsate was added to 9.0ml of a diluent blank (10^{-1}) and vortexed. Serial dilutions from 10^{-1} to 10^{-4} were made and plated onto Petri film to enumerate *Enterobacteriaceae* [2], generic *E. coli* [3], total coliforms [3], and to perform the Aerobic Plate Count (APC) [4].

Salmonella

The rinsates samples were analyzed for the presence of *Salmonella* by adding 30 ml of rinsate to 30 ml BPW and stomaching for two minutes. An aliquot of the homogenate was screened for *Salmonella* using the DuPont BAX system [5] [6]. The level of *Salmonella* in the screen positive samples was estimated using the "Most Probable Number" (MPN) procedure [7]. These samples were used to enrich three 10 ml, three 1 ml and three 0.1 ml samples. 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 expressed as "MPN/ml" and the presence of Salmonella in the positive tubes was confirmed. Those *Salmonella* MPN results where at least one tube was positive for *Salmonella* are labeled as "quantifiable" samples in the data tables of this report.

Campylobacter

To detect and enumerate *Campylobacter*, the rinsate samples were analyzed using two separate methods. A Quantitative Detection and Enumeration method, which was derived from a recommendation from NACMCF [8], developed by USDA/ARS, was used on Post-Chill and Re-Hang rinsate samples. The Qualitative Detection method, which was used only with the rinsates obtained from Post-Chill samples, included an enrichment step.

1. **Qualitative Detection.**

For this analysis, 30ml of the rinsate was mixed with 30ml of Blood Free 2X Bolton's Enrichment Broth 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 and the plates incubated. Colonies that exhibited the characteristic colonial morphology of *Campylobacter* were later confirmed and those samples scored as positive. Plates on which there were no *Campylobacter* colonies were scored as negative.

2. Quantitative Detection and Enumeration.

Rinsates from both Post-Chill and Re-Hang samples were plated directly onto Campy-Cefex agar plates. In order to plate the highest amount of rinsate from those samples in which the levels of *Campylobacter* was expected to be low (Post-Chill samples), 250ul of the Post-Chill rinsate was plated directly onto Campy-Cefex plates. A total of 1ml (250ul on each of four plates) was thus inoculated. A ten-fold dilution of the rinsates was obtained by plating 100ul directly onto Campy-Cefex plates. If necessary, the rinsate would be further diluted with sterile diluent and 0.1ml of the dilution plated directly onto Campy-Cefex plates. After incubation, colonies that exhibited the characteristic colonial morphology of *Campylobacter* were counted and up to 5 colonies of each morphology (if there was more than one) confirmed. These samples were scored as positive and the bacterial counts recorded as colony forming units (CFU) per milliliter (ml) of chicken carcass rinsate. Plates on which there were no *Campylobacter* colonies were scored as negative.

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 immediately 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 scored as negative for both tests.

4. Theoretical Limit of Detection

For the Quantitative Detection and Enumeration method, the maximum amount of the undiluted Post-Chill rinsate analyzed was 1ml, 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 "<1cfu/ml" in this report. For Re-Hang samples, because of a higher concentration of background flora in the rinsate, it was necessary to first dilute the rinsate in sterile diluent 1:10, then 1ml of this diluted sample was plated as described above. The theoretical limit of detection for this assay was ten colonies per ml.

For the Qualitative Detection method, 30 ml of the rinsate was mixed with 30ml of Blood Free 2X Bolton's Enrichment Broth to allow *Campylobacter* to multiply to levels that could be detected by the agar plating and screening procedures. Because this method contains an enrichment step, the actual quantity of *Campylobacter* in the original rinsate cannot be determined. However, the theoretical limit of detection for this assay is one cell in 30ml and samples positive in this test can be expressed as having a *Campylobacter* concentration of >0.03cfu/ml. Samples which were negative for this test were reported to be "<0.03cfu/ml" in this report.

RESULTS

A total of 6,550 samples were collected from young chicken carcasses during this survey. Because only paired samples were processed in the laboratory, there were an equal number of Re-Hang and Post-Chill samples analyzed (3,275). In plants that processed samples during two shifts, rinsate samples were collected during both shifts, as opposed to one rinsate sample per shift in those plants with only a single shift.

Table 1 presents a summary of the test results of samples that were quantified and combines the results from both shifts. In addition, the data has 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. Moreover, arithmetic mean, mean standard error, the geometric mean (with a 95% confidence interval) and the $\log_{10}$ of the geometric mean are also 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, an estimation of the percent positive and a 95% confidence interval is given for the pathogenic organisms.

When the percent positive rates of *Salmonella* were compared between Re-Hang and Post-Chill samples, the percent positive rate was 40.70% vs. 5.19%. When comparing the Re-Hang and Post-Chill samples for *Campylobacter* the percent positive rates were 71.36% vs. 10.66%. These raw numbers should not be considered as the national prevalence for these pathogens but rather the percent positive sample results observed during this survey.

Table 2 reports the percent positive rate (40.23%) for the qualitative *Campylobacter* test results. 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 chicken carcasses at Post-Chill may be too low to detect using this method. During preliminary analysis of chicken rinse samples using only direct plating, this theory was confirmed and it was determined that a qualitative method should be added. During the actual survey, a portion of the rinsate 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.

For Re-Hang samples, 99.9% of the Aerobic Plate Count (35^{o}C) samples were above the LOD for these microorganisms while 99.7% of the samples were above the LOD for *Enterobacteriaceae* microorganisms. The percent of samples above the LOD for coliform microorganisms and generic *Escherichia coli* were 99.7% and 99.2%, respectively (Table 1).

For Post-Chill samples, the percent positive rates were lower than their Re-Hang counterparts. The percent positive rates greater than the LOD for APC (35^{o}C), *Enterobacteriaceae,* coliforms and generic *Escherichia coli* were respectively, 97.1%, 57.4%, 47.8% and 38.7% (Table 1).

For the purpose of finding differences, a comparison of means was done between the means of presence of the organism at Re-Hang and at Post-Chill (Table 1). The statistical analysis (at p-value 0.05) shows that all levels of all the bacterial targets are significantly lower at Post-Chill when compared to the Re-Hang.

In order to estimate prevalences of *Salmonella* and *Campylobacter*, estimation procedures are necessary that account for non-response or differences in proportion of samples and volume (slaughtered chickens) over time. After accounting for these, the national prevalence for *Salmonella* was estimated to be 7.5%, with a standard error of 0.43%. For *Campylobacter*, the estimated prevalence was 46.7% with a standard error of 0.87%. Details of the analysis used for estimating prevalence are presented in a report that can be obtained at http://www.fsis.usda.gov/PDF/Technical_Paper_Performance_Guidance_Broilers.pdf

For Re-Hang, the APC (35^{o}C) were distributed such that 61.1% of the samples contained between 10,001 and 100,000 microorganisms while for Post-Chill samples the APC (35^{o}C) were distributed such that 49.2%, the largest distributed group, was between 101 and 1000 microorganisms.

Similarly, for *Enterobacteriaceae,* coliforms and generic *E. coli* samples, the distribution of these organisms above the LOD was 1 to 2 magnitudes less for Post-Chill samples than the distribution for Re-Hang samples.

For Re-Hang samples, of the 3,275 Post-Chill samples tested for *Campylobacter*, 2,337 were confirmed positive for *Campylobacter* via quantitative analysis with 938 samples below the LOD. Of the quantifiable samples, 438 (13.4%) had a quantitative range from 10 to 100 CFU/ml and 841 (25.7%) samples ranged from 101 to 1000 CFU/ml. The remaining ranges for

Campylobacter are reported in Table 4, with one sample within the highest range, 1,000,001 to 10,000,000 CFU/ml.

For Post-Chill samples, 349 confirmed positive and, as expected, the levels of *Campylobacter* in these samples were much lower. Of the 3,275 Post-Chill samples tested for *Campylobacter*, 2,926 were below the LOD. Of the remaining samples, 199 (6.1%) had a quantitative range from 1-10, 115 (3.5%) had a quantitative range from 11 to 100 CFU/ml, 28 (0.85%) samples ranged from 101 to 1000 CFU/ml, and 7 (0.21%) samples ranged from 1,001 to 10,000 CFU per ml (Table 7).

Fifteen hundred Re-Hang *Salmonella* samples were confirmed positive via the FSIS qualitative analysis. Upon enumeration with the quantitative method , 167 (11.1%) samples were below the LOD. Of the quantifiable samples, 622 (41.5%) ranged from 0.0301 to 0.3 MPN/ml and 506 (33.7%) samples ranged from 0.301 to 3.0 MPN/ml. Eleven samples were above 30 MPN/ml with 4 of the 11 undetermined (Table 5).

As expected there were many fewer Post-Chill samples that confirmed positive. Of the 3,275 samples tested, only 170 (5.19%) were enumerated and none exceeded 30 MPN/ml. Of these, 123 (46.1%) had a quantitative range from 0.0301 to 0.3 MPN/ml, 38 (14.2%) samples ranged from 0.301 to 3.0 MPN/ml and 9 were between 3.01 and 30 MPN/ml (3.3%) (Table 8).

The *Salmonella* serotypes isolated most often from the young chicken samples were Kentucky (703), Heidelberg (216), Typhimurium (138) and Typhimurium (Copenhagen) (117). These serotypes were consistently isolated most often regardless of the shift the sample was taken during or whether it was collected at Re-Hang or Post-Chill.

Tables 9 and 10 relate to data collected from the plants that had both Shifts 1 and 2 (142 establishments) at Re-Hang and Post-Chill . For the purpose of finding differences, a comparison of the average presence of the organisms at Shift 1 Re-Hang and at Shift 2 Re-Hang was performed. This same comparison of averages was done for the Shift 1 Post-Chill and Shift 2 Post-Chill samples. The test used was a t-test of means for two independent distributions. The p-level was computed based on the t-value for the respective comparison. The statistical tests (at p-value 0.05) showed that none of the levels of organisms were significantly different between Shift 1 Re-Hang and Shift 2 Re-Hang or between Shift 1 Post-Chill and Shift 2 Post-Chill.

DISCUSSION

The Nationwide Microbiological Baseline Data Collection Program: Young Chicken Survey was designed to determine the presence and the concentration of selected bacteria on young chicken carcasses produced in federally inspected plants. In 1999 - 2000, FSIS conducted a similar baseline survey for *Salmonella* and generic *E. coli* (Nationwide Young Chicken Microbiological Baseline Data Collection Program [9]). This more recent survey, unlike the 1999 – 2000 survey, included testing for *Campylobacter*, *Enterobacteriaceae*, total aerobic bacteria and coliforms. Technical modifications such as sampling at two process locations and during two production shifts were made, as well as implementing a new *Campylobacter* direct plating method.

In addition to obtaining the percent positive and levels of various bacteria in chicken rinsate 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 chicken carcasses. First shift, for this survey, was defined as the first shift after plant cleanup in which chickens would be slaughtered. It was expected that bacterial levels on chicken carcasses would be lower during first shift, but, as chicken slaughter continued during the day, the levels would increase the longer the samples were collected from cleanup. Our analysis indicated that there was no statistically significant difference in the levels of the bacteria analyzed

between first and second shift, suggesting that at least in these plants, the length of time from clean-up to sample collection does not influence pathogen levels on chicken carcasses.

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 chicken carcasses are removed from the chill tank. We observed a substantial reduction in the number of samples positive for *Salmonella* from Re-Hang to Post-Chill (40.70% vs. 5.19%) and *Campylobacter* (71.36% vs. 10.66%), suggesting that the anti-microbial interventions had an effect. The prevalence estimate for *Salmonella* was 7.5%, with a standard error of 0.43%. For *Campylobacter*, the estimated prevalence was 46.7% with a standard error of 0.87%. Details of the analysis used for estimating prevalence are presented in a report that can be obtained at http://www.fsis.usda.gov/PDF/Technical_Paper_Performance_Guidance_Broilers.pdf.

During this survey, a new method for the analysis of *Campylobacter* was implemented. This method, recommended by the NACMCF, is a direct plating method and enables the direct enumeration of *Campylobacter* from chicken rinsates, thus giving an indication of the actual level of *Campylobacter* contamination of the carcass. In many cases, 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. 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 procedure 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 making this new method for *Campylobacter* detection and enumeration an official analytical method in the Microbiological Laboratory Guidebook.

TABLES

Table 1. Comparison between Quantified Re-Hang and Post-Chill Samples by Microorganism in the 2007 – 2008 Young Chicken Survey

Microorganisms					Levels of Positives				
Indicator Organism [1]	Sample Collected at	Number of Samples Tested	Number of Samples Quantifiable [2]	Percent Positive	Mean (Data units) [4]	Mean Std Error	Geometric Mean	Geo Mean 95% CI	Log 10 of the Geo Mean
Aerobic Plate Count	Re-Hang	3,275	3,273	99.94%	356,635.2	109,992.0	32,302	30,693 - 33,996	4.51
	Post-Chill	3,275	3,179	97.07%	7,011.9	1,627.5	271	254 - 288	2.43
Enterobacteriaceae	Re-Hang	3,275	3,266	99.73%	129,854.1	81,526.6	1,905	1788 - 2028	3.28
	Post-Chill	3,275	1,880	57.40%	820.1	323.9	37	35 - 40	1.57
Total Coliforms	Re-Hang	3,275	3,266	99.73%	10,907.1	1,393.8	1,054	991 - 1121	3.02
	Post-Chill	3,275	1,566	47.82%	283.3	86.4	31	29 - 33	1.49
Generic *Escherichia coli*	Re-Hang	3,275	3,249	99.21%	6,821.6	905.5	635	596 - 675	2.80
	Post-Chill	3,275	1,266	38.66%	123.7	21.8	25.4	24 - 27	1.40
Pathogenic Organism									
Campylobacter [3]	Re-Hang	3,275	2,337	71.36%	9,017.0	883.0	859.0	785 - 940	2.93
	Post-Chill	3,275	349	10.66%	67.0	12.4	9.1	7.5 - 11.9	0.96
Salmonella [5]	Re-Hang	3,275	1,333	40.70%	2.99	0.85	0.42	0.38 - 0.47	-0.36
	Post-Chill	3,275	170	5.19%	0.70	0.14	0.14	0.11 - 0.18	-0.84

(1) Units are CFU/ml
(2) Above the Limit of Detection (LOD)
(3) LOD are different for Re-Hang and Post-Chill because different methods were used
(4) All mean differences between Re-Hang and Post-Chill are statistically significant
(5) *Salmonella* measurements are in MPN/ml

Table 2. Comparison between Qualitative Re-Hang and Post-Chill Samples by Pathogenic Organism in the 2007 – 2008 Young Chicken Survey

Pathogenic Organism	Sample Collected at	Number of Samples	Number of Positives	Percent Positive
Campylobacter	Post-Chill	2,926 (1)	1,177	40.23%
Salmonella	Re-Hang	3,275	1,500	45.80%
	Post-Chill	3,275	267	8.15%

(1) Because this analysis was only performed on samples below the LOD, the already quantified positives (349) were not qualitatively tested. Therefore, the number of samples tested was reduced from 3,275 to 2,926, i.e., 3275 – 349.

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

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
10-100	495	15.11	521	15.9
101-1,000	1,545	47.18	2,066	63.1
1,001-10,000	977	29.83	3,043	92.9
10,001-100,000	204	6.23	3,247	99.1
100,001-1,000,000	26	0.79	3,273	99.9
1,000,001-10,000,000	2	0.06	3,275	100.0
Total	3,249	100.00	-	-

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

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
10-100	438	13.37	1,376	42.0
101-1,000	841	25.68	2,217	67.7
1,001-10,000	719	21.95	2,936	89.6
10,001-100,000	310	9.47	3,246	99.1
100,001-1,000,000	28	0.85	3,274	100.0
1,000,001-10,000,000	1	0.03	3,275	100.0
Total	2,337	100.00	-	-

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

Range, MPN/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
0.0301-0.3	622	41.47	789	52.6
0.301-3.0	506	33.73	1,295	86.3
3.01-30.0	194	12.93	1,489	99.3
30.01-300.0	7	0.47	1,496	99.7
Undetermined (1)	4	0.27	1,500	100.0
Total	1,333	100.00	-	-

(1) Includes 1 value >1,100 and 3 values >11

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

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
10-100	1,109	33.86	3,118	95.2
101-1,000	134	4.09	3,252	99.3
1,001-10,000	21	0.64	3,273	99.9
10,001-100,000	2	0.06	3,275	100.0
Total	1,266	100.00	-	-

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

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
1-10	199	6.08	3,125	95.4
11-100	115	3.51	3,240	98.9
101-1,000	28	0.85	3,268	99.8
1,001-10,000	7	0.21	3,275	100.0
Total	349	100.00	-	-

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

Range, MPN/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
0.0301-0.3	123	46.07	220	82.4
0.301-3.0	38	14.23	258	96.6
3.01-30	9	3.37	267	100.0
Total	170	100.0	-	-

Table 9. Statistical Comparison between Re-Hang Shift 1 and Shift 2 Samples in the 2007 – 2008 Young Chicken Survey

	Sample S-1	Mean at Shift 1	Std Dev at Shift 1	Geo Mean at Shift 1	log 10 of Geo Mean at Shift 1	Sample S-2	Mean at Shift 2	Std Dev at Shift 2	Geo Mean at Shift 2	log 10 of Geo Mean at Shift 2	p-value (**)
Generic *E. coli* (CFU/ml)	1,441	5,285	38,022	588	2.77	1,443	8,444	65,197	630	2.80	**0.89**
***Campylobacter* (CFU / ml)**	1,049	8,118	35,227	794	2.9	1,017	8,866	36,835	871	2.94	**0.36**
***Salmonella* (MPN / ml)**	582	2.01	5.90	0.42	-0.37	610	3.78	44.80	2.42	-0.38	**0.65**

(**) None of the differences between Shift 1 and Shift 2 at Re-hang are statistically significant at p-value 0.05

Table 10. Statistical Comparison between Post-Chill Shift 1 and Shift 2 Samples in the 2007 – 2008 Young Chicken Survey

	Sample S-1	Mean at Shift 1	Std Dev at Shift 1	Geo Mean at Shift 1	log 10 of Geo Mean at Shift 1	Sample S-2	Mean at Shift 2	Std Dev at Shift 2	Geo Mean at Shift 2	log 10 of Geo Mean at Shift 2	p-value (**)
Generic *E. coli* (CFU/ml)	523	54	165	22	1.34	512	82	415	21.4	1.33	**0.85**
***Campylobacter* (CFU / ml)**	127	46	165	7	0.84	130	49	219	6.8	0.83	**0.11**
***Salmonella* (MPN / ml)**	69	0.65	1.61	0.16	-0.78	73	0.55	1.83	0.11	-0.94	**0.27**

(**) None of the differences between Shift 1 and Shift 2 at Post-chill are statistically significant at p-value 0.05

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