United States Department of Agriculture

Food Safety and Inspection Service

Office of Public Health Science

Microbiology Division

The Nationwide Microbiological Baseline Data Collection Program: Raw Chicken Parts Survey

January 2012–August 2012

FOREWORD

This report provides an overview of The Nationwide Microbiological Baseline Data Collection Program: Raw Chicken Parts Survey. The Microbiological Analysis and Data Branch, Science Division, Office of Public Health Science conducted this survey during an 8-month period (January to August 2012) to estimate the percent positive, levels of *Salmonella* and *Campylobacter*, and indicator bacteria on raw chicken parts. FSIS used this information to estimate national prevalence of the two pathogens on raw chicken parts. FSIS inspection personnel in the Office of Field Operations (OFO) collected samples, which were analyzed by Food Safety Net Services, Ltd., San Antonio, TX under contract.

THE NATIONWIDE MICROBIOLOGICAL BASELINE DATA COLLECTION PROGRAM:
RAW CHICKEN PARTS BASELINE SURVEY (RCPBS)

JANUARY 2012–AUGUST 2012

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EXECUTIVE SUMMARY

FSIS conducted the Raw Chicken Parts Baseline Survey (RCPBS) from January 2012 to August 2012. The survey generated 2,496 valid samples of diverse chicken parts collected at the end of the production line. The valid samples were obtained from 449 establishments that produce raw chicken parts under Federal Inspection. The number of samples collected from each establishment ranged from 1 to 17 with an average of 6 samples per establishment. These samples were analyzed to determine the percent positive and pathogen levels for *Salmonella* and *Campylobacter*, as well as the levels for generic *Escherichia coli*, Aerobic Plate Count (APC), *Enterobacteriaceae*, and total coliforms. FSIS compared percent positive and levels of specific microbiological targets to determine if significant differences existed between samples taken from parts with skin-on versus skin-off and between production shifts. Comparisons among types of chicken parts were not conducted.

Summary findings

Qualitative Microbiological Results—the *Salmonella* percent positive rate for chicken parts was 26.3% and 21.4% for *Campylobacter*.
Indicator organisms—the percent positive rates at the end of the production line were 98.84% for Aerobic Plate Count (35^{o}C APC), 96.23% for Enterobacteriaceae, 88.46% for total coliforms, and 62.6% for generic *E. coli*.

Shift 1 versus Shift 2 comparison:
FSIS compared levels in chicken parts products for *Salmonella, Campylobacter,* generic *E. coli,* and APC based on production in first versus second production shift. Of the 123 establishments that were sampled during both shifts, no statistically significant difference (P-value > 0.05) was apparent for any pathogen or indicator bacteria for the percent positive samples or levels of bacterial target.

Skin-on vs. skin-off comparison:
Chicken parts are sold to the consumer with or without skin. It was determined that no significant difference (p-value > 0.05) exist in the percent of *Salmonella* positive samples in parts, whether skin remained intact or was removed (26.2% versus 26.5%). The same was true for generic *E. coli*. However, *Campylobacter* demonstrated significantly (p-value < 0.05) higher counts and percent positives (24.0% vs. 16.6%) in parts with skin-on. For APC the count is significantly higher on chicken parts with skin-off (P-value < 0.05).

***Salmonella* serotypes:**
The top three most frequent *Salmonella* serotypes found in this baseline study were Kentucky (30%), Enteritidis (24.7%), and Typhimurium (20.5%); 26 other serotypes ranging from 0.2% to 7.6% were identified.

National prevalence estimates for *Salmonella* and *Campylobacter*:

FSIS calculated the prevalence or weighted average of *Salmonella* and *Campylobacter* for all chicken parts. These national prevalence estimates are different from the percent positives because they are weighted in relation to production volume.

- The estimated national prevalence of *Salmonella* in chicken parts is 24.02% with a 95% confidence interval between 19.24% and 28.79%.

- The estimated national prevalence of *Campylobacter* in chicken parts is 21.70% with a 95% confidence interval between 18.70% and 24.69%.

INTRODUCTION

The United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) 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 number (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 is weighted by production volume, quantitative pathogen data from this and other baseline studies provide a scientific basis for exposure assessment for use in microbial risk assessments. The baseline survey establishes microbiological criteria for industry performance standards, determines raw chicken parts production parameters, and considers the regional variability in prevalence and levels of pathogen and indicator bacteria. Data collected during baseline surveys is essential for meeting these mission-critical needs.

FSIS conducted the first Raw Chicken Parts Baseline Survey (RCPBS) from January to August 2012. A 90-day training period preceded the survey (shakedown) for the field and laboratory personnel to prepare for the baseline. In addition, FSIS created mailboxes where the Office of Field Operations (OFO) inspection program personnel (IPP) could submit questions about the survey. The preparation process also used formal FSIS Notices to provide IPP information about the survey and instructions for sampling.

During this RCPBS, FSIS implemented the following specifications:

1. Sample raw chicken parts at the end of the production line. The type of part is randomly determined. The end of the production line was selected because it is the point of production closest to the consumer within the establishment after all antimicrobial interventions are completed.

2. Identify the shift when sampling occurred for establishments that reported having two production shifts; sample collectors were instructed to record the shift in the sample form (Shift 1 or Shift 2). In establishments that reported a single production shift, all samples were collected at Shift 1[1].

OBJECTIVES

The Raw Chicken Parts Baseline Survey had six objectives:

1. Collect microbiological data from raw chicken parts rinsate samples to determine the presence and quantitative levels of specific microbiological targets and measure the change over time. Microbiological targets included:

   Pathogens:
   - *Salmonella*
   - *Campylobacter*

   Indicator bacteria:
   - Generic *Escherichia coli*
   - Total Aerobic Plate Count (35 ^{o}C APC)
   - Enterobacteriaceae
   - Total coliforms

[1] Shift 1 is defined as production time that occurred immediately after a pre-operational sanitation inspection was performed.

2. Calculate the national prevalence of *Salmonella* and *Campylobacter* in raw chicken parts using the production volume as reference for weighting the samples. The RCPBS will account for non-response if necessary.

3. Provide data for the calculation of industry performance standards for pathogen contamination of raw chicken parts.

4. Provide data for use in the development of risk assessments, which inform risk management decisions, risk-based sampling programs, and/or regulatory policy decisions.

5. Obtain *Salmonella* isolates to generate serotyping data.

6. Assess the effects of various factors on the microbiological profile (e.g., geographic region, inspection system, plant size, skin-on versus skin-off, and specific antimicrobial interventions).

PROGRAM DESIGN

Establishments Included in the Sampling Frame

During the survey preparation, FSIS computerized database Public Health Information System (PHIS) did not contain chicken parts production volume or plants producing chicken parts. During the shakedown, FSIS identified a partial list of establishments that produce chicken parts and the production volume for those establishments. Inspectors updated current production volume for each establishment during the actual survey by answering questions on the sample request forms. All Federal establishments producing chicken parts that were identified during shakedown were included in the sampling frame and were eligible for selection during the actual survey.

FSIS initially identified 660 establishments likely to produce raw chicken parts in the shakedown phase. These establishments were eligible for the baseline survey and represented all raw chicken parts produced in the United States under Federal Inspection. FSIS removed some establishments from the sampling frame due to lack of raw chicken parts production. The final sampling frame included 464 establishments.

Sample Collection Design

The sample design considered establishment production volume, availability of chicken parts, the nature and number of bacterial targets, sampling logistical limitations, the specific data to be collected, sampling costs, and the collection and analytical methods. FSIS scheduled 5,000 samples to collect in six months. When samples were not collected for lack of production, inspectors, when possible, re-scheduled the collection for other day.

Two types of errors were considered—sampling errors and non-sampling errors. Both sampling and non-sampling errors may affect the reliability of results and had to be considered in designing this survey. Sampling errors occur because observations are derived from a subset of the entire population; non-sampling errors may be attributed to many sources inherent in the collection of samples, laboratory analysis, and processing of data. FSIS considered these types of errors in determining the total sample size and the specific number of samples to be collected from each establishment.

The RCPBS incorporated a multi-stage cluster design that included sampling each establishment through time. Raw chicken parts were selected at frequencies defined by three-production volume. Some establishments operated on two shifts. After assessing the production volume information collected during the survey, FSIS used the following volume categories for sampling weight and calculation of prevalence:

Production Volume Category 1 consisted of large establishments that produce more than 70,000,000 pounds of chicken parts per year. This stratum contains plants that produce 81.0% of the total chicken parts in the sampling frame. The target frequency collection for this stratum was 2 samples per month (16 samples per establishment during the 8-month survey).

Production Volume Category 2 consisted of medium establishments that produce 1,000,000 or more pounds of chicken parts, but fewer than 70,000,000 pounds per year. This stratum contains establishments producing 18.8% of the total chicken parts in the sampling frame. The target frequency collection for this stratum was an average of 1.33 samples per month (10 samples per establishment during the 8-month survey).

Production Volume Category 3 consisted of small establishments that produce less than 1,000,000 pounds of chicken parts per year. This stratum contains establishments producing 0.09% of the total chicken parts in the sampling frame. The target frequency collection for this stratum was an average of 1.33 samples per month (10 samples per establishment during the 8-month survey).

For better sample distribution between shifts, sample collectors were instructed to alternate sample collection between Shift 1 and Shift 2 in those plants that produced chicken parts during two production shifts.

Sampling Location within the Establishment

Inspectors collected raw chicken parts samples at the end of the production line, after all establishment interventions and just prior to packaging for shipment. This is the point of collection in the establishment where product is closest to consumer distribution. FSIS collected samples throughout the 8-month survey from this location in the production line and from both production shifts in establishments with two shifts.

Sample Collection and Description

Samples were aseptically collected by FSIS inspectors following the procedures in FSIS Directive 10,230.5 (2/4/98), instructions provided on the computer-generated sample forms, and specific program's instructions. Inspectors collected a 4-pound bag of raw chicken parts. Raw chicken parts are defined as:

- Raw (uncooked) skin-on or skinless, bone-in or boneless chicken breasts, thighs, wings, legs, necks, backs, half- or quarter-carcasses, and internal organs, such as giblets (e.g., liver, heart, or gizzard), typically available to consumers or for export. Other parts may also be included in the survey.
- RCPBS did not include entire chicken carcasses or chicken parts that have been ground, otherwise comminuted, marinated, breaded, or further processed before shipping from the establishment. See 9 CFR 381.170(b), which details requirements for the specified cuts of poultry.

To accommodate all sample requirements inspectors were instructed to follow the sample collection procedures below:

Sample collection procedure:

1. Randomly select a production line.
2. Use a random number generator to select a type of chicken part.
3. At the end of the production line before packing, aseptically place 4 lbs. of the selected chicken part in a sterile bag provided by FSIS. Only one type of chicken part will go to the bag (e.g., only wings).
4. Pour 400 ml Buffered Peptone Water (BPW) into the sterile bag containing chicken parts and shake vigorously according to instructions provided.
5. Pour the shaken BPW rinsate into the sterile screw-top container provided by FSIS and secure the top tightly.
6. Cool samples (on ice or in refrigerator) within five minutes of collection and keep sample temperature at approximately 4^{o}C, until shipping. Do not freeze.
7. Ship all samples to the lab on the day of collection.

The samples were shipped to the contract laboratory by an overnight delivery service on the same day they were collected or the next day if the sample was collected on the second shift. The samples were collected Monday through Friday during regular operating hours (Monday through Thursdays for second shift). Only those samples received at the laboratory the day after the sample was shipped with a sample receipt

temperature between 0°C to 10°C (inclusive) were analyzed. FSIS discarded samples received outside this temperature range.

SELECTION OF MICROORGANISMS

To obtain microbiological data for use in the development of risk assessments, risk-based sampling programs and/or regulatory policy decisions, the samples were analyzed for *Salmonella* and *Campylobacter* (the pathogens), and for generic *E. coli*, total coliforms, total APC, and *Enterobacteriaceae* (the indicator organisms).

SAMPLE ANALYSIS METHODS

Indicator Bacteria

To analyze the samples for the indicator bacteria, 1 ml of rinsate was added to 9.0 ml 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* (1), generic *E. coli* (2), total coliforms (2), and to perform APC (3).

Salmonella

The rinsate 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 Qualicon BAX system (4) (5). The level of *Salmonella* in the screen positive samples was estimated using the "Most Probable Number" (MPN) procedure (6). 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 estimate low levels of *Salmonella statistically,* and the results were expressed as "MPN/ml". The presence of *Salmonella* in the positive tubes was culture-confirmed by the FSIS MLG method. 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

Two different FSIS testing methodologies were used to detect and enumerate *C. jenjuni, C. coli, and C. lari*:

1) A Quantitative Detection and Enumeration method based on direct plating of small volumes of samples rinsate and dilutions, and
2) An Enrichment-based Qualitative Detection method designed to detect Campylobacter in a larger portion of rinsate. The methodology was originally developed by USDA Agricultural Research Service (ARS) and recommended by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) (7) and subsequently adapted for FSIS baseline testing and laboratory use.

1. Qualitative Detection

For this analysis, 30 ml of the rinsate test portion was mixed with 30 ml 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 detectable levels by screening and agar plating procedures. Following enrichment, a portion of this culture was inoculated onto Campy-Cefex plates, and the plates were incubated. Colonies that exhibited the characteristic colonial morphology of *Campylobacter* were later confirmed by direct microscopic examination for cellular morphology and motility, and serological confirmation as *C. jenjuni, C. coli, and C. lari*. Confirmed samples were scored as positive. The larger test portion used for this method provided greater opportunity for detecting *Campylobacter.*

2. Quantitative Detection and Enumeration

To provide an opportunity for enumeration, sample rinsates were plated directly onto Campy-Cefex agar plates. A total of 1 ml (250 µl on each of four plates) of sample rinsate was plated. For 10^{-1} and additional

dilutions, 100 µl was plated. After incubation, colonies that exhibited the characteristic colonial morphology of *Campylobacter* were counted and up to five colonies of each morphology (if there was more than one) were confirmed. Confirmed samples were scored as positive and the bacterial counts recorded as colony forming units per milliliter (CFU/ml) of chicken parts rinsate.

3. Sequence of analysis

For the analysis of samples, 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 sample was scored as negative for both tests.

4. Theoretical Limits of Detection

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

For the Qualitative Detection method, 30 ml of the rinsate was mixed with 30 ml 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 30 ml and samples positive in this test can be expressed as having a *Campylobacter* concentration of >0.03 CFU/ml. Samples that were negative for this test were reported to be "<0.03 CFU/ml" in this report.

DATA ANALYSIS METHODS

General Overview

The national prevalence calculation estimates *Salmonella* and *Campylobacter* in raw chicken parts. National prevalence provides a national average of expected values for *Salmonella/Campylobacter* on raw chicken parts. Sampling for the survey was designed so the results represent all plants producing raw chicken parts. This approach uses class or "strata" by production volume to define the strata as large, medium, and small. This design ensures that small plants are adequately represented in the study despite their low production volume. However, strata sampling introduces bias in the sample collection. To counterbalance this bias, all plants were weighted using the total national production. After these considerations, the specialized statistical software WesVar v 5.1 (8) was used to calculate the national prevalence estimate and its uncertainty. Details of file development, sample weight calculation, and software processing is presented in the appendix Statistical Analysis Plan (SAP)

RESULTS

RCPBS analyzed 2,496 samples. In plants that processed chicken parts during one shift, FSIS collected one sample per shift. In plants that processed samples during two shifts, FSIS collected samples alternating during both shifts; these samples were approximately evenly distributed between both shifts.

Table 1 presents a summary of the test results of all quantified samples and combines all the results (skin-on, skin-off, chicken part, and shift).

For the raw chicken parts, 98.84% of the APC samples were above the limit of detection (LOD) (LOD <10 CFU/ml) for these microorganisms, while 96.23% of the samples were above the LOD for Enterobacteriaceae. The percent of samples above the LOD for total coliforms and generic *E. coli* were 88.46% and 62.26%, respectively.

Tables for the distribution of pathogenic microorganism levels were assembled for *Salmonella* (Table 2) and *Campylobacter* (Table 3). The distribution of indicator organisms are detailed for APC (Table 4), Enterobacteriaceae (Table 5), Total Coliforms (Table 6), and generic *E. coli* (Table 7). These distributions are presented in ranges of factors of 10.

For *Salmonella* serotyping, the sample's prevailing colony was picked and processed. The results do not necessarily represent the only serotype in the sample. The *Salmonella* serotypes isolated most often in raw chicken parts samples were Kentucky (30%), Enteritidis (24.7%), Typhimurium[2] (20.5%). Table 8 shows the frequencies and percentages calculated for the three dominant *Salmonella* serotypes and 26 other serotypes.

FSIS performed a comparison of the average presence of the microorganisms at Shift 1 to Shift 2. The analyst identified 123 establishments with 2 shifts for this comparison. Inspectors collected a few samples on the third shift but the amount was too small for comparison. Two indicators, generic *E. coli* and APC, and two pathogens, *Salmonella* and *Campylobacter*, were selected for the comparison. Table 9 details this comparison, which indicates no significant difference between shift 1 and 2 for all microorganisms.

Raw chicken parts are usually available to consumers with skin on or off. FSIS conducted a comparison of raw chicken parts to determine differences between these two options. Table 10 presents the comparison using two indicator organisms and two pathogens. There was no significant difference with skin-on versus skin-off samples when chicken parts were tested for generic *E. coli* or *Salmonella.* Chicken parts with skin-on had a significantly higher number of positive results for *Campylobacter* compared to parts with skin off. The indicator APC shows a significantly lower concentration in parts with skin on than parts with skin-off.

Because FSIS tested two pathogens simultaneously during this survey, the question as to what are the chances that a chicken part would be positive for any pathogen could be answered. There were 657 samples (26.32%) positive for *Salmonella* and 534 samples positive (21.39%) for *Campylobacter*. However, the number of samples positive for either pathogen is 1,038 samples, which indicates that 41.58% (1,038/2,496) of chicken parts are positive for one or another of the pathogens. In addition, 153 or 6.13% of raw chicken parts in the survey tested positive for both pathogens simultaneously.

Table 12 shows the percent positive for *Salmonella* and *Campylobacter* by type of chicken part. Chicken neck (55%) is the part with a higher percent positive for both *Salmonella* and *Campylobacter*. Breast samples (16.1%) show the lowest percent positive for *Campylobacter* (13.5% for skinless breast and 25.6% for skin-on breast). Quarter carcass (20.6%) shows the lowest percent positive for *Salmonella*.

FSIS calculated the prevalence or weighted average in relation to production volume for both pathogens. This national prevalence estimate is different from the percent positive, because it is weighted in relation to production volume and adjusted for non-response. Figure 1 shows the WesVar software output window for *Salmonella* and *Campylobacter* prevalence in chicken parts baseline. In summary the results of prevalence are:

- The estimated prevalence of *Salmonella* in chicken parts is 24.02% with a 95% confidence interval between 19.24% and 28.79%.

- The estimated prevalence of *Campylobacter* in chicken parts is 21.70% with a 95% confidence interval between 18.70% and 24.69%.

The survey results will be slightly differ for percent positive rates for *Salmonella* and *Campylobacter* compared to national prevalence (*Salmonella*: percent positive 26.32% vs. national prevalence 24.02%; *Campylobacter*: percent positive 21.39% vs. national prevalence 21.70%). These differences are produced because of the introduction of production volume weighting necessary to compensate for the survey's design bias.

[2] Typhimurium includes var. 5- (formerly var. Copenhagen)

DISCUSSION

RCPBS was designed to determine the presence and the levels of selected bacteria on raw chicken parts produced in federally inspected plants. This is the first baseline survey performed by FSIS on this type of poultry product. In addition, FSIS wanted to determine the national prevalence of *Salmonella* and *Campylobacter* on raw chicken parts. Additional goals for this survey include determining if there was a significant difference between production shifts as it relates to bacterial levels, and comparison the bacterial presence and levels on raw parts with skin-on versus parts with skin-off.

Entire young chicken carcasses are the source of raw chicken parts. FSIS wants to compare the presence and levels of bacteria between these two forms of raw chicken that are purchased by consumers. FSIS has analyzed entire young chicken carcasses in three previous baseline surveys: June 2007–June 2008 (18), November 1999–October 2000, and July 1994–June 1995. In the most recent entire young chicken carcass survey, FSIS obtained results at post-chill, after the application of cleaning interventions and compared these results to raw chicken parts. The comparison shows that chicken parts have a higher percent positive for all indicators except for APC (Table 13). For the pathogens, the percentage positive in chicken parts doubles for *Campylobacter* and is four times higher for *Salmonella* as compared to entire chicken carcasses. The comparison holds true when skin-on chicken parts are compared to whole chicken carcasses (Table 14). The increase in the presence of pathogenic bacteria may be because a single positive carcass may yield several positive parts and further cross contamination resulting from the contaminated carcasses/parts to other parts during processing/handling.

TABLES AND FIGURES

Table 1. Summary for Quantified Samples by Microorganism in the 2012 RCPBS

Microorganisms Indicator Organism (1)	Sample Collected at	Number of Samples Tested	Number of Samples Quantifiable (2)	Percent Positive	Levels of Positives Mean (Data units)	Mean Std Error	Geometric Mean	Geo Mean 95% CI	Log 10 of the Geo Mean
Aerobic Plate Count	End of Production Line	2,496	2,467	98.84%	558,018,333	82,793,270	32,359	(28,225 – 37,393)	4.51
Enterobacteriaceae	End of Production Line	2,496	2,402	96.23%	16,645	2,502	312	(284 – 342)	2.49
Total Coliforms	End of Production Line	2,496	2,208	88.46%	2,544	713	82	(77 – 88)	1.91
Generic Escherichia coli	End of Production Line	2,496	1,554	62.26%	701	265	31	(29 – 37)	1.50
Pathogenic Organism									
Salmonella (3)	End of Production Line	2,496	657 (3)	26.32%	0.82	0.11	0.09	(0.075 – 0.10)	-1.06
Campylobacter	End of Production Line	2,496	534 (4)	21.39%	10.41	2.61	2.09	(1.84 – 2.38)	0.32

(1) Units are CFU/ml

(2) Above Limit of Detection (LOD); LOD = 10 CFU/ml

(3) *Salmonella* measurements are in MPN/ml; LOD = 0.030 MPN/ml

(4) *Campylobacter* measurements are in CFU/ml; LOD = 1 CFU/ml. These are total positives; this is the addition of 335 (13.4%) quantifiable samples plus 199 (7.9%) positives detected by qualitative test.

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Table 2. Distribution of Quantified *Salmonella* Samples in the RCPBS

Range, MPN/ml	Number of Samples[(1)]	Percent of Total	Cumulative Number	Cumulative Percent
< 0.030	201	30.6%	201	30.6%
0.030 - 0.30	316	48.1%	517	78.7%
0.301 - 3.00	102	15.5%	619	94.2%
3.01 - 30.00	38	5.8%	657	100.0%
> 30.01	0	0.0%	657	100.0%
Total	657	100.0%	-	-

LOD < 0.030 MPN/ml
(1) Only positive samples are included regardless if under LOD

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Table 3. Distribution of Quantified *Campylobacter* Samples in the RCPBS

Range, CFU/ml	Number of Samples [(1)]	Percent of Total	Cumulative Number	Cumulative Percent
< LOD	199	37.2%	199	37.3%
1 -10	247	46.3%	446	83.5%
10.01-100	81	15.2%	527	98.7%
100.01-1,000	6	1.1%	533	99.8%
> 1,000	1	0.2%	534	100.0%
Total	534	100.0%	-	-

LOD < 1 CFU/ml
(1) Only positive samples are included regardless if under LOD

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Table 4. Distribution of Quantified APC Samples in the RCPBS

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD < 10	29	1.2%	29	1.2%
10 - 100	23	0.9%	52	2.1%
101 - 1,000	289	11.5%	341	13.6%
1,001-10,000	764	30.6%	1,105	44.2%
10,001-100,000	664	26.6%	1,769	70.8%
100,001-1,000,000	401	16.1%	2,170	86.9%
1,000,001-10,000,000	199	8.0%	2,369	94.9%
10,000,001-100,000,000	32	1.3%	2,401	96.2%
100,000,001-1,000,000,000	25	1.0%	2,426	97.2%
> 1,000,000,000	70	2.8%	2,496	100.0%
Total	2,496	100.0%	-	-

LOD < 10 CFU/ml

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Table 5. Distribution of Quantified *Enterobacteriaceae* Samples in the RCPBS

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD < 10	94	3.8%	94	3.8%
10 - 100	820	32.9%	914	36.6%
101-1,000	1017	40.7%	1,931	77.4%
1,001-10,000	366	14.7%	2,297	92.0%
10,001-100,000	141	5.6%	2,438	97.7%
100,001-1,000,000	57	2.3%	2,495	100.0%
> 1,000,000	1	0.0%	2,496	100.0%
Total	2,496	100.0%	-	-

LOD < 10 CFU/ml

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Table 6. Distribution of Quantified Total Coliform Samples in the RCPBS

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD -10	288	11.6%	288	11.5%
10 - 100	1330	53.3%	1,618	64.8%
101-1,000	731	29.3%	2,349	94.1%
1,001-10,000	108	4.3%	2,457	98.4%
10,001-100,000	26	1.0%	2,483	99.5%
100,001-1,000,000	12	0.5%	2,495	100.0%
> 1,000,000	1	0.0%	2,496	100.0%
Total	2,496	100.0%	-	-

LOD <10 CFU/ml

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Table 7. Distribution of Quantified Generic *Escherichia coli* Samples in the RCPBS

Range, CFU/ml	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
< LOD	942	37.8%	942	37.8%
10 -100	1316	52.8%	2,258	90.6%
101-1,000	198	7.9%	2,456	98.5%
1,001-10,000	31	1.2%	2,487	99.7%
10,001-100,000	6	0.2%	2,493	99.9%
100,001-1,000,000	3	0.1%	2,496	100.0%
> 1,000,000	0	0.0%	2,496	100.0%
Total	2,496	100.0%	-	-

LOD < 10 CFU/ml

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Table 8. *Salmonella* Serotypes for RCPBS

***Salmonella* Serotypes**		
Serotype Name	**Count**	**Percentage**
Kentucky	197	30.0%
Enteritidis	162	24.7%
Typhimurium*	135	20.55%
Heidelberg	50	7.6%
Thompson	28	4.3%
Schwarzengrund	17	2.6%
4,[5],12:i:-	16	2.4%
Infantis	11	1.7%
8,20:-:z6	4	0.6%
Montevideo	4	0.6%
Braenderup	3	0.45%
Newport	3	0.45%
Johannesburg	2	0.3%
Mbandaka	2	0.3%
4,12:Nonmotile	1	0.15%
6,7:-:1,5	1	0.15%
6,7:-:e,n,z15	1	0.15%
8,20:i:-	1	0.15%
Agona	1	0.15%
Brandenburg	1	0.15%
Duisburg	1	0.15%
Give	1	0.15%
Kiambu	1	0.15%
Muenchen	1	0.15%
Ohio	1	0.15%
Ouakam	1	0.15%
Rough_O:f,g,s:-	1	0.15%
Senftenberg	1	0.15%
Uganda	1	0.15%
Multiple Serotypes	8	1.2%
Total	**657**	**100.00%**

* Typhimurium includes var. 5- (formerly var. Copenhagen)

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Table 9. Statistical Comparison between Shift 1 and Shift 2 Samples in the RCPBS

A Goodness-of-Fit test was conducted on the data to see if the source distribution was normal. The Shapiro-Wilk "W" test rejected the hypothesis of normality at p> 0.0001; consequently, a non-parametric Wilcoxon/Kruskal-Wallis test (Rank Sums) was conducted in all cases (17)

Indicators Pathogens	Sample at Shift 1	Mean at Shift 1	Mean St Error at Shift 1	Geo Mean at Shift 1	Log10 of Geo Mean at Shift 1	Sample at Shift 2	Mean at Shift 2	Mean Sd Error at Shift 2	Geo Mean at Shift 2	Log10 Geo Mean at Shift 2	p-value (*)
Generic *E. coli* (CFU/ml)	322	995	757	34.28	1.53	279	88	18	29.51	1.47	p = 0.22
APC (CFU/ml)	462	2.1×10^8	1.0×10^8	9,051	3.95	376	1.2×10^8	7.2×10^7	9,243	3.96	p = 0.81
Salmonella (MPN/ml)	113	0.73	0.22	0.10	-0.99	107	0.87	0.25	0.09	-1.03	p = 0.46
Salmonella (Percent Positive)		0.24%					0.28%				p = 0.18**
Campylobacter	118	8.87	1.89	2.39	0.38	79	11.89	4.22	2.46	0.39	p = 0.89
Campylobacter (Percent Positive)		0.25%					0.21%				p = 0.16**

(*) Non-parametric Wilcoxon / Kruskal-Wallis test (Rank Sums) at α = 0.05 between shift 1 vs. shift 2 (123 establishments with 2 shifts) for actual values.
(**) Test of proportions for percent positives

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Table 10. Statistical Comparison between Skin-on vs. Skin-off for Samples in the RCPBS

A Goodness-of-Fit test was conducted on the data to see if the source distribution was normal. The Shapiro-Wilk "W" test rejected the hypothesis of normality at p> 0.0001; consequently, a non-parametric Wilcoxon/Kruskal-Wallis test (Rank Sums) was conducted in all cases (17)

Indicators Pathogens	Sample Skin-on	Mean Skin-on	Mean St Error Skin-on	Geo Mean Skin-on	Log10 of Geo Mean Skin-on	Sample Skin-off	Mean Skin-off	Mean Sd Error Skin-off	Geo Mean Skin-off	Log10 Geo Mean Skin-off	p-value (*)
Generic *E. coli* (CFU/ml)	983	233	86	31.08	1.49	571	1,506	704	32.01	1.50	p = 0.93
APC (CFU/ml)	1,574	4.2×10^8	8.9×10^7	20,836	4.31	893	7.9×10^8	1.6×10^8	71,076	4.85	p = 0.0001
Salmonella (MPN/ml)	418	0.84	0.15	0.08	-1.06	239	0.79	0.16	0.09	-1.06	p = 0.96
Salmonella (Percent Positive)	1,595	26.20%				901	26.52%				p = 0.88**
Campylobacter	384	11.2	3.51	2.29	0.35	150	8.33	2.37	1.66	0.22	p = 0.015
Campylobacter (Percent Positive)	1,595	24.07%				901	16.64%				p = 0.0001**

(*) Non-parametric Wilcoxon / Kruskal-Wallis test (Rank Sums) at $\alpha = 0.05$ between skin-on vs. skin-off for actual values.
(**) Test of proportions for percent positives

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Table 11. Non-response Rate and Percent Positive by Strata for Samples in the RCPBS

Strata	Discarded Samples	Valid Samples	Total Samples	Percent of Discarded	Percent Positive by Strata	
					Salmonella	*Campylobacter*
1	179	629	808	22.2%	27.9%	25.5%
2	359	1,000	1,359	26.4%	26.6%	22.6%
3	322	867	1,189	27.1%	24.8%	16.9%
Total/p-value	860	2,496	3,356	P= 0.04*	P = 0.37**	P = 0.0001***

(*) p = 0.04 There is significant difference in percent discarded samples by strata.
(**) p = 0.37 There is no significant difference in *Salmonella* percent positive by strata.
(***) P = 0.0001 There is significant difference in *Campylobacter* percent positive by strata.

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Table 12. *Salmonella* and *Campylobacter* Percent Positive by Specific Chicken Part for Samples in the RCPBS

Chicken Part by Type	Number of Samples	Number of *Salmonella* Positives	Number of *Campylobacter* Positives	Percent *Salmonella* Positives	Percent *Campylobacter* Positives
A - Breast	**776**	**210**	**125**	**27.06%**	**16.11%**
B - Neck	**22**	**12**	**12**	**54.55%**	**54.55%**
C - Leg	**584**	**141**	**119**	**24.14%**	**20.38%**
D - Wing	**321**	**107**	**75**	**33.33%**	**23.36%**
E - Half Carcass	**149**	**33**	**29**	**22.15%**	**19.46%**
F - Quarter Carcass	**330**	**68**	**92**	**20.61%**	**27.88%**
G - Giblets	**57**	**23**	**25**	**40.35%**	**43.86%**
H - Other	**248**	**59**	**55**	**23.79%**	**22.18%**
NP*	**9**	**4**	**2**	**44.44%**	**22.22%**
Totals	**2,496**	**657**	**534**		

(*) Part type not provided

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Table 13. **Comparison of Bacteriological Results for Young Chicken Baseline Survey (YCBS) 2007–2008 at Post-chill vs. Raw Chicken Parts Baseline Survey (RCPBS) 2012, in Percent Positives.**

	Salmonella	*Campylobacter*	APC	Enterobacteriaceae	Coliforms	Generic *E. coli*
YCBS	5.9	10.6	97.0	57.4	47.8	38.6
RCPBS	26.3	21.4	98.8	96.2	88.4	62.2

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Table 14. **Comparison of Bacteriological Results for Young Chicken Baseline Survey (YCBS) 2007–2008 at Post-chill vs. Raw Chicken Parts (with skin-on only) Baseline Survey (RCPBS) 2012, in Percent Positives.**

	Salmonella	*Campylobacter*	APC	Enterobacteriaceae	Coliforms	Generic *E. coli*
YCBS	5.9	10.6	97.0	57.4	47.8	38.6
RCPBS Skin-on	26.3	24	98.6	94.4	85.5	61.6

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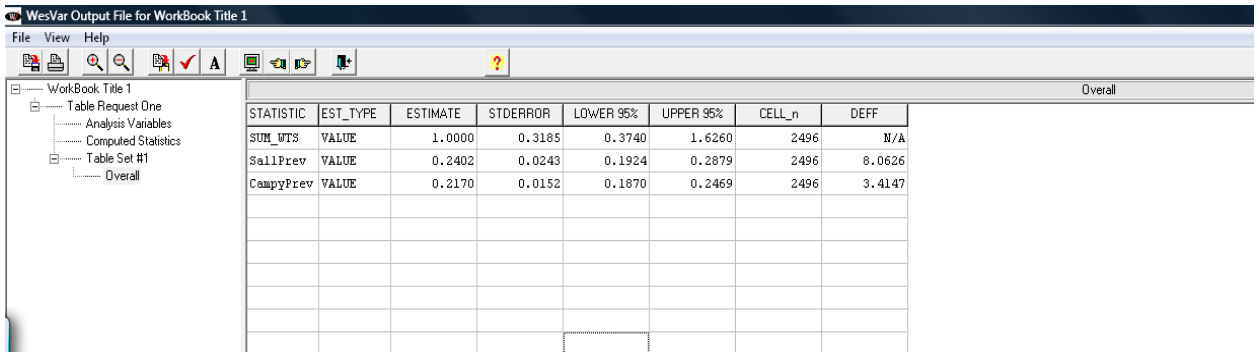
Figure 1. WesVar Output Window for *Salmonella* and *Campylobacter* Prevalence in Chicken Parts Baseline.



STATISTIC	EST_TYPE	ESTIMATE	STDERROR	LOWER 95%	UPPER 95%	CELL_n	DEFF
SUM_WTS	VALUE	1.0000	0.3185	0.3740	1.6260	2496	N/A
SallPrev	VALUE	0.2402	0.0243	0.1924	0.2879	2496	8.0626
CampyPrev	VALUE	0.2170	0.0152	0.1870	0.2469	2496	3.4147

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APPENDIX
Statistical Analysis Plan (SAP)

Work Flow Overview:

To calculate summary tables and national prevalence estimates, FSIS processed the data during the study in the following steps:

1. Managed existing files to update total volume production of raw chicken parts during the survey period (8 months).

2. Verified the final data set after completing the survey. Shipping date and collection date for each sample were checked to ensure they were no more than one day apart. The Agency confirmed that sample receipt temperatures were within analyzable limits and verified answers to supplemental questions on the sampling form. FSIS determined whether *Salmonella* had appropriate MPN values and MPN positive tube combinations. The laboratories obtained serogroup and serotype information for all *Salmonella* positive samples. FSIS identified outliers for indicator organisms to verify if they were correct measurements, review the data file, and corrected data entry errors.

3. Calculated general statistics, tested comparison hypothesis for pathogens and indicators, and assembled the results in tables.

4. Merged existing files containing information about volume production to determine total production, calculated establishment and sample weight, and adjusted for non-responses or missing samples. The analyst prepared sample files for special software processing. JMP (17) statistical software was used.

5. Analyzed sample files using the software "WesVar v 5.1" (8) to obtain point estimates and uncertainty.

Data

The original raw data file for RCPBS contains production information collected during the survey, answers to supplemental questions on the sample collection form, and lab results. Production information is essential for calculating the annual production and is used to calculate weight of each establishment. This file contains general information including:

a. Volume information on 464 establishments in this study. Inspectors sampled these establishments; however, some of these establishments have no representation because all samples from them were discarded. Valid samples were collected on 449 establishments.
b. FSIS calculated additional information about stratification and production by stratum.
c. The individual sample weight was calculated by integrating the production file with the survey results.
d. Other sections of the file showed establishment information including, plant identification number, state, stratification calculations, etc.
e. FSIS assembled a final file with valid results for calculation of the presence and concentration of microorganisms; 2,496 samples produced conclusive results.
f. Sample collectors' answers to questions posted on block 28 of the sample collection form.

Calculation of Base Sample Weights

The scope of the sampling design for the RCPBS divided the qualified producing establishments into three classes or strata. Collecting an unequal number of samples from pre-determined groups implies that the sample collection is not completely random, so the establishments do not have an equal probability of selection (9). As such, some

sectors of the population were sampled at a higher frequency, and this type of design can introduce bias. To counter-balance the bias, each sample is weighted to account for its relative impact on the result. A way to relate the sample results and their uncertainty to all of the establishments producing raw chicken parts and to estimate parameters is by using special statistical software (discussed in "WesVar Statistical Procedures" section). However, before the application of the software the weight of each sample was calculated.

The base weight of a sampled unit is the reciprocal of its probability of selection into the sample (9, 10). The weight acts as an equalizer representing the sampling units that were not selected. In mathematical notation, if a unit is included in the sample with probability *Pi,* then its base weight, denoted by W_i, is given by

$$W_i = 1/P_i$$

The base weights in the multi-stage RCPBS must reflect the probabilities of selection at each stage. In the case of a two-stage design, the *j-th* Primary Sampling Unit (PSU*,* the establishment*)* is selected with probability P_j at the first stage, and the *i-th* (bag of chicken parts) is selected with probability *pi(j)* at the second stage. Then the overall probability of selection of every unit in the sample is given by

$$P_{ij} = P_j * P_{i(j)}$$

And the base weight is the reciprocal

$$W_i = 1/P_{ij}$$

In case of a simple non-stratified sample, the weight (in relation to production volume) is $V_j / \Sigma V_j$ or volume of plant *"j"* divided by total production (all plants). In case of a two-stage stratified survey (like the chicken parts), each stratum is treated as an independent sample and the basic weight of an establishment (PSU) in stratum *"j"* is

$$W_p = (V_j / \Sigma V_{sj}) * (V_{ij} / \Sigma V_j)$$

Where:

V_j is the volume of stratum *j* <u>including establishments not sampled</u>
$\sum V_{sj}$ is the volume of establishments that were <u>sampled</u> in stratum *"j"*
V_{ij} is the volume of establishment "*i*" in stratum "*j*"
Σv_j is total volume of establishments in the frame, <u>sampled or not</u>

Because the study's design calls for multiple samples drawn from individual establishments, the greater the number of samples taken from an establishment is, the smaller the individual sample weight for that establishment. As such, samples take shares of the weight of the establishment. In view of this fact, the weight for an individual sample is:

$$W_{ij} = 1/n_{ij} * (V_j / \Sigma V_{sj}) * (V_{ij} / \Sigma V_j) \qquad (1)$$

Where:

n_{ij} is the number of samples taken in plant *"i"* in stratum *"j"*

Corrections for Non-response

It is rarely the case that all desired information is obtained from all sampled units. For instance, some samples may be discarded because of temperature deviations, or the establishment was not producing the product at collection time. This type of missing information is called unit non-response and may create bias in the estimate (10 11).

If there are systematic differences (non-random) between the respondents and non-respondents among strata, then estimates based solely on the respondents may be biased.

The size of the non-response bias for a sample mean is a function of two factors:

- The proportion of the population that does not respond by strata.
- The size of the difference in population means among strata.

If the proportions of response in the strata are not significantly different, then there is no need for adjustment, because the same proportion of samples is missing in each stratum. If the population mean of interest has no significant difference among strata, there is no need for adjustment. If one of the above conditions is not satisfied, then there is a need to adjust for non-response.

Table 11 provides a summary of the response rate per stratum and the percent positive per stratum for both pathogens. Stratum 1 shows a disproportionally and statistically significant (p = 0.04) lower number of missing samples, and Stratum 3 for *Campylobacter* shows a disproportionally and statistically significant (p = 0.0001) lower number of percent positives. This indicates that there is a need for adjustment for non-response.

Essentially, the adjustment for non-response transfers the base weights, previously calculated, of all eligible non-responding sampled units to the responding units. This transfer is implemented in the following steps:

1. Compute the response rates for each stratum;
2. Use the reciprocal of the stratum response rates for non-response adjustments; and
3. Calculate the non-response adjusted weight for the *j-th* establishment as:

$$W_j = W_{1j} * W_{2j}$$

Where:
W_{1i} is the base weight (formula 1)
W_{2i} is the non-response adjustment

Furthermore, no analysis for seasonality was conducted because the study lasted for only eight months. In addition, a test of multiple proportions by month (12) showed that percent positives by month are not significantly different for *Salmonella* (p-value = 0.55). Because there are no significant differences by month for *Salmonella* in chicken parts, no further adjustments was made.

The adjusted weight was calculated with the information in the data files and variables developed using previously defined formulas. After additional preparation, the analyst assembled a WesVar ready–to-use file.

WesVar Statistical Procedures (13)

When data are collected as part of a complex sample survey, analytically there is often no easy way to produce unbiased design-consistent estimates of variance. The variances of survey statistics, including means and proportions that are estimated using standard statistical packages, are usually inappropriate and are often too small. Replication methods provides a method to estimate variance for the types of complex sample designs and weighting procedures like the one encountered in this study.

The basic idea behind replication is to select subsamples repeatedly from the whole sample, calculate the statistics of interest for each subsample, and then use these subsamples or replicates to estimate the variance of the full sample statistics. The subsamples are called replicates and the statistics calculated from these replicates are called replicate estimates. Because of the weighting and the application of the replication method, the outcome obtained in the sampling can be extended to the entire U.S. operation as a national prevalence measurement. The replication methods and theory used in this survey derive from the computer statistical package WesVar version 5.1 (13). The package provides several methods of replication, including the Balance Repeated Replication (BRR) and the Jackknife procedures (JKs). For the particular design of the sample at hand with many establishments or Primary Sampling Unit (PSU) per stratum, the methodology selected was the Jack Knife (n).

One of the main advantages of replication is its ease of use at the analysis stage. The same estimation procedure is used for the full sample and for each replicate. The variance estimates are then readily computed by a simple procedure. Furthermore, the same procedure is applicable to most statistics, such as means, percentages, ratios, correlations, etc. These estimates can be calculated for analytic groups or sub-populations. Another important advantage of replication is that it provides a simple way to account for adjustments that are made in weighting (13, 14, 15, 16).

WesVar accomplishes the implementation of the replication methods in four steps.

Step 1 WesVar divides the sample into subsample replicates that mirror the design of the sample by specifying the variance of the variables strata and PSU.

Step 2 WesVar calculates weights for each replicate, following the same procedures used for the full-sample weight. The replicate weights are attached to the WesVar data file.

Step 3 The software calculates replicate estimates for each of the replicates using the same methods used for the full sample estimate.

Step 4 WesVar estimates the variance of the full-sample estimate, using the resulting full-sample and replicate estimates. The outputs of the program reflect this computation.

The next step calculates the replicated weights. The WesVar program accomplished this by using the variables strata, already in file, (the division of plants by size, 1 to 3) and a new variable PSU. The analyst created the variable PSU (establishments) by allocating a number (1 to n) to each PSU in each stratum; this allowed for the partition of the sample into subsample replicates that mirrored the design of the sample. With the introduction of the variables weights, strata, and PSU, the file was finally ready for processing in WesVar.

Calculation of *Salmonella* National Prevalence in Raw Chicken Parts

Figure 1 shows the WesVar output with results for *Salmonella* and *Campylobacter.* Because of the use of replicated weight, this result extends to the entire universe of chicken parts in the U.S. market.

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