United States Department of Agriculture

Food Safety and Inspection Service

Office of Public Health Science

Microbiology Division

The Nationwide Microbiological Baseline Data Collection Program: Market Hogs Survey August 2010 – August 2011

FOREWORD

This report provides an overview of The Nationwide Microbiological Baseline Data Collection Program: Market Hogs Survey and discusses the microbiological results from this study conducted for thirteen months from August 2010 to August 2011. 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 market hog carcasses, as well as to estimate the national prevalence of *Salmonella* in market hogs. 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 FSIS inspection personnel in the Office of Field Operations (OFO). The microbiological analyses of the survey samples were conducted by a contract laboratory − Food Safety Net Services, Ltd., San Antonio, TX.

THE NATIONWIDE MICROBIOLOGICAL BASELINE DATA COLLECTION PROGRAM: MARKET HOGS SURVEY AUGUST 2010 – AUGUST 2011

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EXECUTIVE SUMMARY

The Market Hogs Baseline Survey (MHBS) was conducted from August 2010 to August 2011. FSIS collected 3,920 sponge samples from market hog carcasses (1,960 at Pre-Evisceration and 1,960 at Post-Chill from 2 separate shifts when available) at 152 establishments that slaughtered market hogs under Federal Inspection. These samples were analyzed to estimate the percent positive rate and levels of *Salmonella*, generic *Escherichia coli*, Aerobic Plate Count (APC), *Enterobacteriaceae*, and total coliforms. The presence and levels of specific microbiological targets were compared to determine if significant differences existed between samples taken at pre-evisceration and post-chill and between production shifts.

Pre-evisceration vs. post-chill:
The *Salmonella* percent positive rate at pre-evisceration was 69.64%, whereas at post-chill it was reduced to 2.70% (significant with P-value < 0.05). The indicator organism percent positive rates at pre-evisceration vs. post-chill were: Aerobic Plate Count (35°C APC) 99.70% vs. 98.41%, *Enterobacteriaceae* 98.11% vs. 24.20%, Total coliforms 97.19 vs. 17.65%, and generic *E. coli* 95.81% vs. 11.78%. (Table 1)

Shift1 vs. Shift 2:
Comparison by shift and location was performed for *Salmonella* and generic *E. coli*. At pre-evisceration, there was no statistically significant difference (P > 0.05) in *Salmonella* levels between shift 1 and shift 2. However, there were statistical differences in generic *E coli*, i.e., higher level in shift 2 than in shift 1. At post-chill, there were not enough *Salmonella* positive results to make a determination and there was no statistical difference (P > 0.05) for generic *E. coli* between shift 1 and 2. (Table 8)

Salmonella serotypes:
For serotyping, the sample's prevailing colony was picked and further processed, but it should not be assumed that this represents the only serotype in the sample. The most frequent *Salmonella* serotype at pre-evisceration was Derby with 364 occurrences (26.7%), while 77 other serotypes were identified at frequencies ranging from 8.80% to 0.10%. The most frequent *Salmonella* serotype at Post-Chill was Derby with 9 occurrences (17%), while 20 other serotypes were identified at different frequencies ranging from 11.30% to 1.90%. (Tables 7 and 7A)

Salmonella National Prevalence Estimate:
FSIS calculated the prevalence or weighted average at post-chill in relation to production volume for *Salmonella*. This national prevalence estimate is different from the percent positive because it is weighted in relation to production volume.

The estimated prevalence of *Salmonella* in Market Hogs is 1.66% with a 95% confidence interval between 0.82% and 2.51%.

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 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 standards, determines market hogs production parameters, and considers 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.

The FSIS performed two previous baseline surveys on Market Hogs. The first survey was from April 1995 to March 1996 and the second from June 1997 to May 1998. The first survey reported a *Salmonella* percent positive of 8.7% and the second survey reported a *Salmonella* percent positive of 6.9%, both at post-chill. This is the third survey August 2010 to August 2011 and the percent positive for *Salmonella* at post-chill is 2.70%.

During the 2010–2011 baseline survey, FSIS conducted a 90-day training period for the field and laboratory personnel. In addition, FSIS created mailboxes where OFO inspection program personnel could submit questions about the survey. It also used formal FSIS Notices and training DVDs to provide in plant personnel (IPP) information about the survey and instructions for sampling.

During this Market Hogs baseline survey, FSIS implemented the following specifications:

1. Sampling market hog carcasses at two points during processing: Pre-Evisceration and Post-Chill. **Pre-evisceration** refers to the location early in the process prior to evisceration of the hog. **Post-chill** refers to a later point in the process after carcasses are chilled, all interventions completed, and before carcasses enter coolers.

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].

[1] Generally, Shift 1 is defined as the time 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).

OBJECTIVES

The Market Hogs Baseline Survey had six objectives:

1. Collect microbiological data from market hog sponge samples to determine the presence and concentration of specific microbiological targets and measure their change through time. Microbiological targets included:

   Pathogens:
   - *Salmonella*

   Indicator bacteria:
   - Generic *Escherichia coli*
   - Total Aerobic Plate Count (35°C APC)
   - *Enterobacteriaceae*
   - Coliforms

2. Calculate the prevalence of *Salmonella* in market hogs using the production volume as reference for weighting the samples.

3. Calculate pathogen contamination industry performance standards.

4. Assess the effect of the slaughter process on microbiological contamination by comparing the prevalence and quantitative levels of the selected bacteria between market hog carcasses at Pre-Evisceration and Post-Chill.

5. 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).

6. Obtain *Salmonella* isolates to generate sub-typing and antimicrobial resistance data.

PROGRAM DESIGN

Establishments Included in the Sampling Frame

Federal establishments identified in the FSIS Public Health Information System (PHIS) that slaughtered a minimum of 500 hogs in the previous year from May 1, 2009 to April 30, 2010 were included in the sampling frame and eligible for selection during this baseline survey.

Approximately 247 establishments identified in the FSIS Electronic Animal Disposition Reporting System (eADRS) slaughtered market hogs in the year prior to the survey. These establishments were eligible for the Market Hogs Baseline Survey and contributed 99.94% of the total heads of market hogs slaughtered in the U.S. under Federal Inspection from May 1, 2009 to April 30, 2010. Establishments

were removed from the frame due to inspection withdrawal or included in the frame because of new eligible plants being identified with increased production volume. The final sampling frame included 253 establishments.

Sample Collection Design

The factors considered in the design of this survey were size and variability of market hog slaughter plants, the nature and number of bacterial targets, sampling logistical limitations, the specific data to be collected, sampling costs, and the collection and analysis methods.

Two types of errors were considered—sampling errors attributable to sample size and non-sampling errors, such as laboratory methodology. Both sampling and non-sampling errors may affect the reliability of results and had to be considered in designing this program. 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. 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 Market Hogs Microbiological Baseline Survey incorporated a multi-stage cluster design that included sampling each establishment over time. At each establishment in the sampling frame, individual hog carcasses were selected at frequencies defined by five production volume categories. Some establishments operated on two shifts from which inspectors could collect a sample. Samples were collected at two points in the slaughter process: pre-evisceration and post-chill. The following volume categories were used to assign sample collection frequency:

Production Volume Category 1 consisted of large establishments that produce more than 3,000,000 hogs per year. This stratum contains 13 plants that produce 61.6% of the total hogs slaughtered in the sampling frame. Carcass sponges were collected six times per month (72 sampling events per establishment per year) from establishments in this category.

Production Volume Category 2 consisted of medium-large establishments that produce more than 1,000,000 hogs per year, but fewer than 3,000,000 hogs per year. This stratum contains 14 establishments that produce 31.6% of the total hogs slaughtered in the sampling frame. Carcass sponges were collected five times per month (60 sampling events per establishment per year) from establishments in this category.

Production Volume Category 3 consisted of medium establishments that produce more than 30,000 hogs per year, but less than 1,000,000 hogs per year. This stratum contains 30 establishments that produce 6.1% of the total hogs slaughtered in the sampling frame. Carcass sponges were collected once months (12 sampling events in an establishment per year) from establishments in this category.

Production Volume Category 4 consisted of small establishments that produce more than 1,880 hogs per year, but less than 30,000 hogs. This stratum contains 64 establishments that produce 0.5% of the total hogs slaughtered in the sampling frame. Carcass sponges were collected once

every four months (3 sampling events in an establishment per year) from establishments in this category.

Production Volume Category 5 consisted of very small establishments that produce more than 500 hogs per year, but less than 1,880 hogs per year. This stratum contains 126 establishments that produce 0.1% of the total hogs slaughtered in the sampling frame. Carcass sponges were collected once every four months (3 sampling events in an establishment per year) from establishments in this category.

After randomly assigning the shift (Shift 1 or 2) for collection of the first sample in an establishment, subsequent sample requests occurred during the alternate shift. 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 and to maintain consistency, the shift was defined according to shift slaughter totals in eADRS.

The authors of this baseline study estimated that at least 5,016 carcass sponges would need to be collected during 2,508 sampling events[2] per year to ensure a minimum level of precision of ± 1% with 95% confidence, 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 sponges were collected and sampled at two points in the slaughter process; pre-evisceration and post-chill. Sponges were collected throughout the year from both locations in the production chain and from multiple production shifts in establishments with two shifts.

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 pre-evisceration market hog carcass and one post-chill market hog carcass from the same grow-out house was sampled. To accommodate all required analyses, two sides from a single carcass was sampled at each of these points in process using a single sampling sponge for each side. For each sampling sponge, three sites (ham, belly, jowl) on one side of the carcass were sampled using a 100 cm^2 template to represent a total of 300 cm^2 of carcass surface area. Once the samples were collected, the sponges were sealed in zipper lock bags and placed in an insulated shipping container with frozen gel packs capable of maintaining the proper chilling temperature for transport. 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

[2] A sampling event consists of one pre-evisceration sponge and one post-chill sponge being collected concurrently, but samples were collected from two different carcasses in each sampling event.

slaughter operations (Monday through Thursdays for second shift). Only those samples received at the laboratory the day after sample collection, with a sample receipt temperature between 0°C to 15°C (inclusive), were analyzed. Samples received outside this temperature range were discarded.

SELECTION OF MICROORGANISMS

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 *Salmonella* (pathogen), generic *E. coli*, total coliforms, total Aerobic Plate Count (35°C APC), and *Enterobacteriaceae* (indicator organisms).

SAMPLE ANALYSIS METHODS

Indicator Bacteria

One of the two sponge samples collected at each point in process was adjusted to a total Buffered Peptone Water (BPW) diluent volume of 25 ml; 1 ml of diluent was diluted using 9.0 ml of fresh BPW a diluent blank (10^{-1} dilution) and vortexed. Serial dilutions from 10^{-1} to 10^{-4} were plated onto the appropriate Petrifilm™ followed AOAC-validated protocols to enumerate *Enterobacteriaceae* [(1)], generic *E. coli* [(2)], total coliforms [(2)], and 35°C Aerobic Plate Count (APC) [(3)]. The resulting limit of detection (LOD) adjusted to CFU/cm^2 was 0.83.

Salmonella

The second carcass sponge sample collected at each point in process (i.e., the opposite side of the carcass from the indicator sample sponge) was analyzed as prescribed in the FSIS Microbiological Laboratory Guidebook (MLG) *Salmonella* chapter in place at the time (i.e., MLG 4C.02 and 4.04 prior to January 2011 and MLG 4C.03 and 4.05 thereafter). Method revision did not impact sample results. The sponge samples were prepared for *Salmonella* analysis by adding each sponge to 50 ml BPW and hand massaging for two minutes. Enriched BPW was screened for *Salmonella* using the DuPont BAX system [(4)]. Using the overnight refrigerated reserve diluent from the indicator sample sponge, the levels of *Salmonella* in the screen positive samples were estimated by the "Most Probable Number" (MPN) procedure [(6)], testing three 1 ml, three 0.1 ml and three 0.01 ml aliquots. The pattern of positive and negative results among these individual qualitative tests was used to estimate levels of *Salmonella*, and the results were expressed as "MPN/cm²" (LOD = 0.025 MPN/cm^2). The presence of *Salmonella* in the screen-positive qualitative and MPN enrichments was confirmed using the MLG culture isolation and identification method [(5)]. 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.

DATA ANALYSIS METHODS

General Overview

This section presents the calculations of the national prevalence estimate of *Salmonella* in market hogs. Estimation of National Prevalence equates to the calculation of a national average of expected values of *Salmonella* on raw market hog carcasses. Because the aim of the survey is to represent all plants producing market hogs, the survey's sampling was statistically designed; this includes the creation of plant's class or "strata" (five strata: extra-large-size, large-size, medium-size, small-size and very-small-size establishments). The design used in this study ensures that small plants have a sizable representation in the study despite their low production volume. However, the introduction of strata sampling introduces bias in the sample collection and needs to be compensated. To counterbalance this bias, all plants were weighted using the total national production. After all these considerations, the specialized statistical software WesVar v 5.1[(7)] was used to calculate the national prevalence estimate and its uncertainty.

Statistical Analysis Plan (SAP)

Work Flow Overview:

The data during the study were processed in the following steps to calculate national prevalence estimates.

1. Manage existing files to update total volume production of market hogs during the survey period (13 months).

2. Clean the final data set after completing the survey. All samples were compared for appropriate shift numbers and adjusted based on project codes or shift numbers provided on forms. This process compared ship date and collect date for each sample to ensure they were the same. In addition, FSIS compared received date and ship date to ensure they were only one day apart, determined whether companion forms matched (pre-evisceration and post-chill). In addition, the Agency confirmed that sample receipt temperatures were within analyzable limits. FSIS determined whether pathogenic targets have appropriate MPN values and MPN positive tube combinations. The Agency obtained serogroup and serotype information for all *Salmonella* positive samples, as well as identified outliers for indicator organism numbers. Finally, FSIS corrected data entry errors.

3. At the end of the survey, the analyst merged existing files containing information about volume production to determine total production, calculate plant weight and sample weight, and adjust non-responses to sample weight. The analyst prepared sample files for special software processing.

4. FSIS obtained the point estimates and uncertainty values by analyzing the sample files using the available software package. In this study, the analyst used "WesVar v 5.1" for the analysis.

Data

The MHBS data is contained in two files—production information collected during the survey and lab results of data collected for *Salmonella*. A brief file description follows:

1. Production File: This file contains information essential for calculating the total production for the 13-month period, which is crucial to calculate plant weight. This file contains establishment information prior to the survey including:
    a. Volume information on all the establishments that slaughter market hogs and are under Federal Inspection in the USA that were considered for this survey.
    b. Volume information on 253 of the total establishments that were eligible for the survey. Plants that slaughtered fewer than 500 heads per year were excluded.
    c. Additional information about stratification and production by stratum was calculated in this file.
    d. This file was later merged with the Survey Results File to calculate the individual sample weight.

2. Survey Results File: This file contains information on scheduled and collected samples and includes:
    a. Establishment information including, plant IDs, states, etc.
    b. Stratification calculations.
    c. Sample (4,429) scheduling and collection; each sample was analyzed for the presence and concentration of microorganisms, including *Salmonella* and indicator organisms. From this initial amount, 3,920 samples produced conclusive results (1,960 samples at Pre-Evisceration and 1,960 samples at Post-Chill).
    d. Answers to questions posted on block 28 provided by inspectors.

Calculation of Base Sample Weights

The scope of the sampling design for the MHBS divided the qualified producing establishments into five classes or strata (for the exact definition of plant class by volume see appendix A). 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 [8]. As such, some sectors of the population sampled in the baseline were sampled at a higher frequency, and this type of design can introduce results bias. To counter-balance the bias, each sample is weighted to account for its relative impact on the result. To properly interpret the sample results and their uncertainty to the entire universe of Market Hog producing plants, parameter estimation requires special statistical methodology (discussed in "Statistical Procedures" section).

The base weight of a sampled unit is the reciprocal of its probability of selection into the sample[8,9]. 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 MHBS must reflect the probabilities of selection at each stage. In the case of a two-stage design in which the *j-th Primary Sampling Unit (PSU, the Establishment)* is selected with probability P_j at the first stage, and the *i-th* (market hog carcass) is selected within a selected PSU 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 market hogs), 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>
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", and
Σv_j is total volume of establishments in the frame, <u>sampled or not</u>

Given that the study's design calls for multiple samples drawn from individual establishments, the greater the number of samples taken from an establishment the smaller the individual sample weight results because 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 [9,10].

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.
- 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 9 provides a summary of the response rate per stratum and the percent positive per stratum. Stratum 5 (very-small establishments) shows a disproportional and statistically significant number of missing samples, and Stratum 3 (medium establishments) shows a disproportionate and statistically significant number of percent positives. This shows that there is a need for adjustment for no-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) and
W_{2i} is the non-response adjustment

Furthermore, the analyst investigated seasonality by conducting a test of multiple proportions by month [(11)]. The test showed that percent positives by month are not significant different from each other (p-value = 0.53). In addition, a test of multiple proportions of percent positives by season (4 groups containing 3 months of data each − winter, spring, summer and fall) was done. The test showed that percent positives by seasons are not significant different from each other (p-value = 0.52). Because there is no significant seasonality of *Salmonella* in Market Hogs, there is no need to adjust for seasonality.

With the information in the two initial data files and previously defined formulas, the adjusted weight was calculated. After additional preparation, a WesVar ready–to-use file was assembled.

WesVar Statistical Procedures [(12)].

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. A technique called replication methods provides the 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[12] version 5.1., which 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 (PSUs) 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 desired, 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[12,13,14,15].

WesVar accomplishes the implementation of the replication methods in four steps. They are:

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, using 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 was to calculate the replicated weights. The WesVar program accomplished this by using the variables strata, already in file, (the division of plants by size, 1 to 5) and a new variable PSU. The variable PSU was created by allocation of 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 Market Hogs

Figure 1 shows the WesVar output window with results for *Salmonella*. Because of the use of replicated weight, this result extends to the entire universe of plants slaughtering Market Hogs in the United States.

RESULTS

A total of 3,920 sponge samples were collected and analyzed from market hogs carcasses during this survey. The laboratory processed paired samples, so 1,960 pre-evisceration and 1,960 post-chill samples were analyzed. In plants that processed samples during only one shift, one sponge sample per shift was collected. In plants that processed samples during two shifts, sponge samples were collected during both shifts.

Table 1 presents a summary of the test results of all samples that were quantified and combines the results from both shifts during pre-evisceration and post-chill. For indicator organisms, the table provides the number of samples quantified, number of positive samples, and percent positive obtained. Moreover, arithmetic mean, mean standard error, the geometric mean (with a 95% confidence interval), and the $\log_{10}$ of the geometric mean are provided. At the bottom of the table, an estimation of the percent positive and a 95% confidence interval is given for *Salmonella*.

When comparing the pre-evisceration and post-chill samples for *Salmonella*, the percent positive rates were 69.64% vs. 2.70%. These raw numbers should not be considered as the national prevalence for this pathogen, but rather the percent positive sample results observed during this survey. The national prevalence estimate for *Salmonella* is presented in section 2 “Calculation of Prevalence” of this report.

For pre-evisceration samples, 99.70% of the Aerobic Plate Count samples were above the limit of detection (LOD) for these microorganisms, while 98.11% of the samples were above the LOD for *Enterobacteriaceae*. The percent of samples above the LOD for coliforms and generic *E. coli* were 97.19% and 95.81, respectively (Table 1).

For post-chill samples, the percent positive rates were lower than their pre-evisceration counterparts. Table 1 shows the percent positive rates greater than the LOD for APC (98.41%), *Enterobacteriaceae* (24.20%), coliforms (17.65%), and generic *E. coli* (11.78%).

FSIS performed a comparison of means of concentration between the means of presence of the organisms at pre-evisceration and at post-chill (Table 1) e.g., APC comparison between 141,766,055 cfu/cm² (log: 8.15) at pre-evisceration and 37,076 cfu/cm² (log: 4.57) at post-chill. 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 pre-evisceration.

Tables for the distribution of microorganism levels were assembled for pre-evisceration and post-chill for *Salmonella* (Table 2 and 2A), APC (Tables 3 and 3A), Enterobacteriaceae (Tables 4 and 4A), Total Coliforms (Tables 5 and 5A) and generic E. coli (Tables 6 and 6A). These distributions are presented in ranges of factor of 10.

For the serotyping the sample’s prevailing colony was picked and further processed, but this does not represent the only serotype in the sample. The *Salmonella* serotypes isolated most often found in

market hog carcass samples at post-chill were Derby (9), Anatum (6), Typhimurium var 5 (6); four other serotypes with frequency 3 were identified. Table 7 shows the frequencies and percentages calculated for each serotype. At pre-evisceration, the serotype Derby (364) was a 26.6%, while Typhimurium var 5 (120) came second with 8.8% of the total. Table 7A shows a complete distribution and percentages of the serotypes.

For the purpose of finding differences for *Salmonella* and generic *E. coli*, a comparison of the average presence of the organisms at Shift 1 pre-evisceration and at Shift 2 pre-evisceration was performed. 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 [16]. Tables 8 and 8A relate to data collected from the plants at pre-evisceration and post-chill that had both Shifts 1 and 2 (13 establishments) and establishes if there are differences.

FSIS calculated the prevalence or weighted average at post-chill in relation to production volume for *Salmonella*. This national prevalence estimate is different from the percent positive, because it is weighted in relation to production volume and adjusted for non-response.

The estimated prevalence of *Salmonella* in Market Hogs is 1.66% with a 95% confidence interval between 0.82% and 2.51%.

DISCUSSION

The MHBS was designed to determine the presence and the levels of selected bacteria on market hog carcasses produced in federally inspected plants. In addition to obtaining the percent positive and levels of various bacteria in market hog sponges, additional goals for this survey include determining if there was a significant difference between production shifts as it relates to bacterial levels on market hog carcasses. For the purpose of this survey, first shift was defined as the shift after plant cleanup in which hogs would be slaughtered. It has been observed in other baselines that bacterial levels on hog carcasses would be lower during first shift and as slaughter continued during the day, the levels of bacteria would increase. FSIS analysis indicated that there were mixed results. At pre-evisceration levels of generic *E. coli* were significantly higher during the second shift, while no difference was observed in the levels of *Salmonella*. At post-chill, levels of generic *E. coli* were different between the shifts. There were not enough positive samples to make a valid comparison for *Salmonella* at post-chill.

A second goal of this survey was to determine the bacterial load reduction (CFU/cm^2) between pre-evisceration and post-chill (Table 1). FSIS expects a substantial reduction because of the various anti-microbial interventions applied to market hog carcasses prior to post-chill. The survey shows substantial reduction in the number of samples positive for *Salmonella* from pre-evisceration to post-chill (69.64% vs. 2.70%); other microorganisms also show significant reductions. This suggests that the antimicrobial interventions had the intended effect.

TABLES AND FIGURES

Table 1. Comparison between Quantified Pre-Evisceration and Post-Chill Samples by Microorganism during the 2010–2011 MHBS.

Indicators	Sample Collection Point	Samples Analyzed	Samples Quantifiable	Percent Positive Samples	Mean Data Units (1) (2) (3)	Mean Std Error	Geometric Mean	Geo Mean (95% CI)	Log10 Geo Mean
Aerobic Plate Count	Pre-evisceration	1,960	1,956	99.70%	141,766,055	88,133,510	645,654.00	575,440 – 724,435	5.81
	Post-chill	1,960	1,929	98.41%	37,076.30	14,890.70	107.15	93.3 - 120.2	2.02
Enterobacteriaceae	Pre-evisceration	1,960	1,932	98.11%	2,487,259	1,157,468	1,023.29	891.2 - 1,174.9	3.01
	Post-chill	1,960	474	24.20%	1,672.50	512.6	5.75	4.57 - 7.24	0.76
Total Coliforms	Pre-evisceration	1,960	1,905	97.19%	2,391,080	1,230,148	831.76	741.3 - 954.9	2.92
	Post-chill	1,960	346	17.65%	1,452.60	569.8	5.37	4.17 - 7.08	0.73
Generic *E. coli*	Pre-evisceration	1,960	1,878	95.81%	1,416,236	836,828.40	602.56	537.0 - 676.0	2.78
	Post-chill	1,960	231	11.78%	871.3	444.9	4.67	3.46 - 6.31	0.67
Pathogen									
***Salmonella*[(4)]**	Pre-evisceration	1,960	1,365	69.64%	8.7	0.99	0.45	0.39 - 0.52	-0.35
	Post-chill	1,960	53	2.70%	2.81	2.11	0.04	0.02 - 0.07	-1.39

(1) Indicator levels are CFU/cm² (i.e., 300 cm^2 sponge with 25 ml diluent yields 12 cm^2 per analyzed ml)
(2) Above LODs are different for Pre-Evisceration and Post-Chill because different dilution ranges were plated for analysis.
(3) All mean differences between Pre-Evisceration and Post-Chill are statistically significant
(4) *Salmonella* levels are MPN/cm^2 (i.e., 300 cm2 sponge with 25 ml diluent yields 12 cm2 per analyzed ml)

Table 2. Distribution of Quantified *Salmonella* - Pre-Evisceration Samples MHBS

Range, MPN/cm^2 (1)	Number of Samples(2)	Percent of Total	Cumulative Number	Cumulative Percent
<0.025	**357**	**26.2%**	**357**	**26.2%**
0.025-0.25	**189**	**13.8%**	**546**	**40.0%**
0.251-2.50	**401**	**29.4%**	**947**	**69.4%**
2.51-25.0	**325**	**23.8%**	**1,272**	**93.2%**
25.01-250.0	**92**	**6.7%**	**1,364**	**99.9%**
>250	**1**	**0.1%**	**1,365**	**100.0%**
Total	**1,365**	**100.0%**	**-**	**-**

LOD < 0.025

(1) Salmonella levels are MPN/cm^2 (i.e., 300 cm^2 sponge with 25 ml diluent yields 12 cm^2 per analyzed ml)

(2) All positive samples are included regardless if under LOD

Table 2A. Distribution of Quantified *Salmonella* – Post-Chill Samples MHBS

Range, MPN/cm^2 (1)	Number of Samples(2)	Percent of Total	Cumulative Number	Cumulative Percent
<0.025	**38**	**71.7%**	**38**	**71.7%**
0.025-0.25	**3**	**5.7%**	**41**	**77.4%**
0.251-2.50	**9**	**17.0%**	**50**	**94.3%**
2.51-25.0	**2**	**3.8%**	**52**	**98.1%**
25.01-250.0	**1**	**1.9%**	**53**	**100.0%**
>250	**0**	**0.0%**	**53**	**100.0%**
Total	**53**	**100.0%**	**-**	**-**

LOD < 0.025

(1) Salmonella levels are MPN/cm^2 (i.e., 300 cm^2 sponge with 25 ml diluent yields 12 cm^2 per analyzed ml)

(2) All positive samples are included regardless if under LOD

Table 3. Distribution of Quantified APC - Pre-Evisceration Samples MHBS

Range, CFU/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD -10	5	0.3%	5	0.3%
10.1 - 100	2	0.1%	7	0.4%
101-1,000	0	0.0%	7	0.4%
1,001-10,000	69	3.5%	76	3.9%
10,001-100,000	339	17.3%	415	21.3%
100,001-1,000,000	783	40.0%	1198	61.3%
1,000,001-10,000,000	562	28.7%	1760	90.0%
> 10,000,000	196	10.0%	1956	100.0%
Total	1,956	100.0%	-	-

LOD < 0.83

Table 3A. Distribution of Quantified APC – Post-Chill Samples MHBS

Range, CFU/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD -10	374	19.4%	374	19.4%
10.1 - 100	641	33.2%	1015	52.6%
101-1,000	592	30.7%	1607	83.3%
1,001-10,000	226	11.7%	1833	95.0%
10,001-100,000	64	3.3%	1897	98.4%
100,001-1,000,000	26	1.3%	1923	99.7%
>1,000,000	6	0.3%	1929	100.0%
Total	1,929	100.0%	-	-

LOD < 0.83

Table 4. Distribution of Quantified Enterobacteriaceae - Pre-Evisceration Samples MHBS

Range, CFU/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD -10	120	6.2%	120	6.2%
10.1 - 100	220	11.4%	340	17.6%
101-1,000	550	28.5%	890	46.1%
1,001-10,000	702	36.3%	1592	82.4%
10,001-100,000	309	16.0%	1901	98.4%
100,001-1,000,000	15	0.8%	1916	99.2%
1,000,001-10,000,000	7	0.4%	1923	99.5%
> 10,000,001	9	0.5%	1932	100.0%
Total	1,932	100.0%	-	-

LOD < 0.83

Table 4A. Distribution of Quantified Enterobacteriaceae – Post-Chill Samples MHBS

Range, CFU/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD -10	336	70.9%	336	70.9%
10.1 - 100	76	16.0%	412	86.9%
101-1,000	37	7.8%	449	94.7%
> 1,001	25	5.3%	474	100.0%
Total	474	100.0%	-	-

LOD < 0.83

Table 5. Distribution of Quantified Total Coliforms - Pre- Evisceration Samples MHBS

Range, CFU/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD -10	126	6.6%	126	6.6%
10.1 - 100	238	12.5%	364	19.1%
101-1,000	619	32.5%	983	51.6%
1,001-10,000	655	34.4%	1638	86.0%
10,001-100,000	226	11.9%	1864	97.8%
100,001-1,000,000	27	1.4%	1891	99.3%
> 1,000,000	14	0.7%	1905	100.0%
Total	1,905	100.0%	-	-

LOD < 0.83

Table 5A. Distribution of Quantified Total Coliforms – Post-Chill Samples MHBS

Range, CFU/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD -10	247	71.4%	247	71.4%
10.1 - 100	56	16.2%	303	87.6%
101-1,000	27	7.8%	330	95.4%
> 1,000	16	4.6%	346	100.0%
Total	346	100.0%	-	-

LOD < 0.83

Table 6. Distribution of Quantified Generic *E. coli* Pre-Evisceration Samples MHBS

Range, CFU/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD -10	116	6.2%	116	6.2%
10.1-100	292	15.5%	408	21.7%
101-1,000	668	35.6%	1076	57.3%
1,001-10,000	585	31.2%	1661	88.5%
10,001-100,000	190	10.1%	1851	98.6%
100,001-1,000,000	15	0.8%	1866	99.4%
1,000,001-10,000,000	7	0.4%	1873	99.8%
>10,000,000	5	0.3%	1878	100.0%
Total	1,878	100.0%	-	-

LOD < 0.83

Table 6A. Distribution of Quantified Generic *E. coli* Post-Chill Samples MHBS

Range, CFU/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
LOD - 10	165	71.4%	165	71.4%
10.1 -100	42	18.2%	207	89.6%
101-1,000	15	6.5%	222	96.1%
1,001-10,000	4	1.7%	226	97.8%
10,001-100,000	5	2.2%	231	100.0%
Total	231	100.0%	-	-

LOD < 0.83

Serotype Name	Count	Percentage	Serotype Name	Count	Percentage
Derby	364	26.70%	412:i:-	3	0.20%
Typhimurium var 5-	120	8.80%	Altona	3	0.20%
Infantis	101	7.40%	Bovismorbificans	3	0.20%
Agona	90	6.60%	Livingstone	3	0.20%
Anatum	88	6.40%	Orion	3	0.20%
London	55	4.00%	310:Nonmotile	2	0.10%
Johannesburg	49	3.60%	Alachua	2	0.10%
Heidelberg	30	2.20%	Anatum var. 15+	2	0.10%
Ohio	30	2.20%	Krefeld	2	0.10%
Uganda	29	2.10%	Reading	2	0.10%
Senftenberg	28	2.10%	Rough_O:-:15	2	0.10%
Brandenburg	26	1.90%	Rough_O:fg:-	2	0.10%
Adelaide	23	1.70%	Rough_O:i:12	2	0.10%
Typhimurium	23	1.70%	Rough_O:Nonmotile	2	0.10%
Multiple Serotypes	21	1.50%	1323:z:-	1	0.10%
Schwarzengrund	21	1.50%	310:eh:-	1	0.10%
Bredeney	20	1.50%	310:lv:-	1	0.10%
Muenchen	19	1.40%	310:lz13:-	1	0.10%
Mbandaka	17	1.20%	319:-:z27	1	0.10%
Montevideo	15	1.10%	4,12:Nonmotile	1	0.10%
Worthington	14	1.00%	40:b:-	1	0.10%
Saintpaul	13	1.00%	67:-:15	1	0.10%
4512:i:-	11	0.80%	67:Nonmotile	1	0.10%
Give	11	0.80%	Albany	1	0.10%
Rissen	9	0.70%	Chailey	1	0.10%
Panama	8	0.60%	Enteritidis	1	0.10%

Uganda_var._15+	8	0.60%	Falkensee	1	0.10%
Cerro	7	0.50%	Give_var._15+	1	0.10%
Liverpool	7	0.50%	Hadar	1	0.10%
Muenster	7	0.50%	Kiambu	1	0.10%
Meleagridis	6	0.40%	Minnesota	1	0.10%
Kentucky	5	0.40%	Muenster_var._15+34+	1	0.10%
Litchfield	5	0.40%	Multiple Serotypes,Uganda	1	0.10%
Manhattan	5	0.40%	Oranienburg	1	0.10%
Newport	5	0.40%	Ouakam	1	0.10%
Nonviable	5	0.40%	Rough_O:d:lw	1	0.10%
Berta	4	0.30%	Rough_O:lvenz15	1	0.10%
Braenderup	4	0.30%	Rough_O:r:15	1	0.10%
Havana	4	0.30%	Thompson	1	0.10%
			Total	**1365**	**100.00%**

Table 7. *Salmonella* Serotypes Identified at Pre-Evisceration in the MHBS

Table 7A. *Salmonella* Serotypes Identified at Post-Chill in the MHBS

Serotype Name	Count	Percentage
Derby	9	17.00%
Anatum	6	11.30%
Typhimurium var 5-	6	11.30%
Adelaide	3	5.70%
Infantis	3	5.70%
Muenchen	3	5.70%
Multiple Serotypes	3	5.70%
Agona	2	3.80%
Brandenburg	2	3.80%
Bredeney	2	3.80%
Montevideo	2	3.80%
Senftenberg	2	3.80%
Typhimurium	2	3.80%
Enteritidis	1	1.90%
Heidelberg	1	1.90%
Johannesburg	1	1.90%
Livingstone	1	1.90%
London	1	1.90%
Ohio	1	1.90%
Oranienburg	1	1.90%
Rissen	1	1.90%
Total	**53**	**100.00%**

Table 8. Statistical Comparison between Pre-Evisceration Shift 1 and Shift 2 Samples in the MHBS

	Sample Shift 1	Mean Shift 1	Std Dev Shift 1	Geo Mean Shift 1	Log10 Mean Shift 1	Sample Shift 2	Mean Shift 2	Std Dev Shift 2	Geo Mean Shift 2	Log10 Geo Mean Shift 2	p-value (*)
Generic *E. coli* (CFU/cm^2)	388	106,612	1,562,871	1,071.50	3.03	370	1,294,299	24,434,668	510.5	2.7	0.0001
***Salmonella* (MPN/cm^2)**	293	6.57	18.77	0.42	-0.36	292	4.86	13.87	0.35	-0.45	0.39

(*) For Generic *E.coli* there is a significant difference between Shift 1 and 2; for *Salmonella* no significant difference was detected at p-value 0.05

Table 8A. Statistical Comparison between Post-Chill Shift 1 and Shift 2 Samples in the MHBS

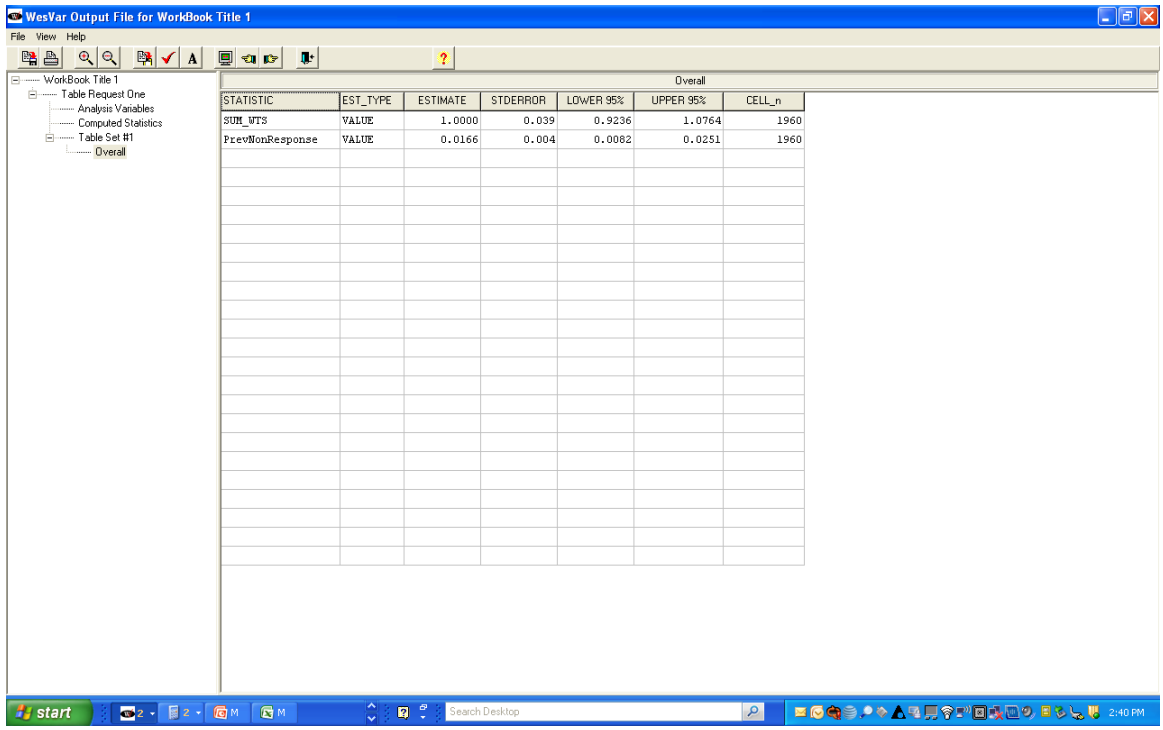
	Sample at Shift 1	Mean Shift 1	Std Dev Shift 1	Geo Mean Shift 1	Log10 Geo Mean Shift 1	Sample at Shift 2	Mean Shift 2	Std Dev Shift 2	Geo Mean Shift 2	Log10 Geo Mean Shift 2	p-value (*)
Generic *E. coli* (CFU/cm^2)	27	670	3,463	1.91	0.28	30	29.5	103.3	2.93	0.46	0.051
***Salmonella* (MPN/cm^2)**	5	2.36	4.85	0.11	-0.95	1	-	-	-	-	-

(*) For Generic *E.coli*, there is not a significant difference between Shift 1 and 2 at p-value 0.05; for *Salmonella*, there is no sufficient samples.

Table 9. Non-response rate and percent positive by strata – Post-Chill

Strata	Lost Samples	Good Samples	Total Samples	% of Discarded Samples	% of Positive Samples
1	91	800	891	10.21%	0.75%
2	44	689	733	6.00%	1.74%
3	39	270	309	12.62%	10.00%
4	22	106	128	17.19%	5.66%
5	51	95	146	34.93%	2.11%

Figure 1. WesVar Output Window for *Salmonella* prevalence in market hogs.



APPENDIX A

Plants in the sampling frame were grouped into five distinctive groups. The grouping was based on slaughter data as reported in the FSIS Electronic Animal Disposition Reporting System (e-ADRS).

Extra-Large-size plants (stratum 1). Plants that slaughtered as least 3,000,000 heads in a 12-month period before the survey.

Large-size plants (stratum 2). Plants that slaughtered as least 1,000,000 heads but less than 3,000,000 heads in a 12-month period before the survey.

Mid-size plants (stratum 3). Plants that slaughtered as least 30,000 heads but less than 1,000,000 heads in a 12-month period before the survey.

Small-size plants (stratum 4). Plants that slaughtered as least 1,880 heads but less than 30,000 heads in a 12-month period before the survey.

Very-Small-size plants (stratum 5). Plants that slaughtered as least 500 heads but less than 1,880 heads in a 12-month period before the survey.

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