United States Department of Agriculture

Food Safety and Inspection Service

Office of Public Health Science

Science Staff

The Nationwide Microbiological Baseline Data Collection Program: Beef-Veal Carcass Survey

August 2014 – December 2015

FOREWORD

This report provides an overview of The Nationwide Microbiological Baseline Data Collection Program: Beef-Veal Carcass Survey. The Science Staff, Office of Public Health Science conducted this survey from August 2014 to December 2015 in order to estimate the percent positive of samples for pathogens – *Salmonella, Escherichia coli* O157:H7, non-O157 Shiga Toxin-Producing *E. coli* (non-O157 STEC) and indicator bacteria on beef and veal carcasses. FSIS used this information to estimate national prevalence of *Salmonella*, *Escherichia coli* O157:H7 and non-O157 STEC on beef and veal carcasses. FSIS inspection personnel in the Office of Field Operations (OFO) collected the samples, which were analyzed by FSIS Field Services Laboratories.

THE NATIONWIDE MICROBIOLOGICAL BASELINE DATA COLLECTION PROGRAM:
BEEF AND VEAL CARCASS BASELINE SURVEY (BVCBS)

AUGUST 2014– DECEMBER 2016

TABLE OF CONTENTS

THE NATIONWIDE MICROBIOLOGICAL BASELINE DATA COLLECTION PROGRAM:
BEEF AND VEAL CARCASS BASELINE SURVEY (BVCBS)

AUGUST 2014– DECEMBER 2015

EXECUTIVE SUMMARY

FSIS conducted the Beef-Veal Carcass Baseline Survey (BVCBS)[1] from August 2014 to December 2015. The statistical design (1) divided the beef establishments in 3 strata according to production volume (1-large, 2-medium, and 3-small); the veal establishments were not divided in strata. The survey generated 2,736 samples of beef and 548 samples of veal. Carcass samples were collected at two points of the slaughter process, 1,368 samples of beef and 274 samples of veal at post-hide removal, and 1,368 samples of beef and 274 samples of veal at pre-chill. Samples at these two points were collected, when possible, from the same carcass; one half of the carcass was sampled at post-hide-removal and the other half at pre-chill. These samples were obtained from 149 establishments under federal inspection: 137 establishments that slaughtered only cattle to produce beef, 10 establishments that slaughtered only veal calves, and two establishments that slaughtered to produce both beef and veal. For beef, the overall number of samples collected from each establishment ranged from 1 to 33 with an average of 10 samples per establishment. For veal, the overall number of samples collected from each establishment ranged from 3 to 33, with an average of 23 samples per establishment. These samples were analyzed to determine the percent positive and quantifiable levels of *Salmonella*, *Escherichia coli* O157:H7, non-O157 Shiga toxin-producing *E. coli* (non-O157 STEC) as well as the percent positive for and quantifiable levels of generic *Escherichia coli*, Aerobic Count (AC), *Enterobacteriaceae*, and total coliforms. FSIS compared percent positive with levels of microbiological targets to determine if significant differences existed between samples taken from post hide-removal and pre-chill for both beef and veal carcasses. In addition, national prevalence (the weighted average based on production) for *Salmonella, E. coli* O157:H7, and non-O157 STEC in both product classes was determined.

Summary of Results

Beef

Qualitative Microbiological Results—the *Salmonella* percent positive for beef carcasses at post-hide-removal was 27.12%, for *E. coli* O157:H7 was 1.83% and 6.14% for non-O157 STEC. *Salmonella* percent positive for beef carcass at pre-chill was 3.36%, 0.66% for *E. coli* O157:H7, and 0.73% for non-O157 STEC.

Indicator organisms— The quantifiable (above limit of detection) percent positive at post-hide-removal was 99.48% for Aerobic Count, 74.67% for *Enterobacteriaceae*, 67.50% for total coliforms, and 75.75% for generic *E. coli*. The quantifiable percent positive at pre-chill was 80.50% for Aerobic Count, 22.40% for *Enterobacteriaceae*, 16.76% for total coliforms, and 13.82% for generic *E. coli*.

***Salmonella* serotypes from beef samples**

For beef at post-hide-removal there were 371 samples positive for *Salmonella* and there were 46 different serotypes identified. The top three most frequent *Salmonella* serotypes isolated from samples collected at post-hide-removal were: Montevideo (21.56%), Anatum (15.90%), and Cerro (10.78%).

At pre-chill, the survey found 46 samples positive for *Salmonella* and there were 20 different serotypes identified. The top three most frequent *Salmonella* serotypes isolates from samples collected at pre-chill were Montevideo (17.38%), Muenchen (10.87%), and I 4,[5],12:i:- and Agona (both with 8.70%).

[1] General industry classifications for veal are: bob veal calves (< 150lbs or 3 weeks of age or less), formula-fed veal calves (151-400lbs), non-formula-fed veal calves (151-400lbs), and heavy calves (> 400lbs).

Beef National prevalence estimates for *Salmonella, E. coli* O157:H7 and non-O157 STEC

FSIS calculated the prevalence or weighted average of *Salmonella, E. coli* O157:H7 and non-O157 STEC for beef carcasses at pre-chill. These national prevalence estimates tend to be different from the percent positive because prevalence estimates are weighted by production volume.

- The estimated national prevalence of *Salmonella* on beef carcasses is 0.72% (95.00% Confidence Interval: 0.21% and 1.21%)
- The estimated national prevalence of *E. coli* O157:H7 on beef carcasses is 0.06% (95.00% Confidence Interval: 0.00% and 0.15%)
- The estimated national prevalence of non-O157 STEC on beef carcasses is 0.21% (95.00% Confidence Interval: 0.00% and 0.51%)

Veal

Qualitative Microbiological Results—the *Salmonella* percent positive for veal carcasses at post-hide-removal was 12.04%, 0.73% for *E. coli* O157:H7, and 23.72% for non-O157 STEC. The *Salmonella* percent positive for veal carcasses at pre-chill was 1.82%, 0.73% for *E. coli* O157:H7, and 9.85% for non-O157 STEC.

Indicator organisms— The quantifiable (above limit of detection) percent positive at post-hide-removal was 99.63% for Aerobic Count, 73.72% for *Enterobacteriaceae*, 66.42% for total coliforms, and 70.44% for generic *E. coli*. The quantifiable percent positive at pre-chill was 75.00% for Aerobic Count, 32.97% for *Enterobacteriaceae*, 31.02% for total coliforms, and 32.48% for generic *E. coli*.

***Salmonella* serotypes from veal samples**

For veal at post-hide-removal there were 33 samples positive for *Salmonella* and there were 14 different serotypes identified. The top three most frequent *Salmonella* serotypes isolated from samples collected at post-hide-removal were: Cerro (21.21%), Montevideo (12.12%), and Newport (12.12%).

At pre-chill there are five samples positive for *Salmonella* containing 3 different serotypes. The three *Salmonella* serotypes found at pre-chill were Newport (40.00%), Typhimurium (40.00%), and Anatum (20.00%).

Veal National prevalence estimates for *Salmonella, E. coli* O157:H7 and non-O157 STEC

FSIS calculated the prevalence or weighted average of *Salmonella, E. coli* O157:H7 and non-O157 STEC for veal carcasses at pre-chill. These national prevalence estimates tend to be different from the percent positive because they are weighted in relation to production volume.

- The estimated national prevalence of *Salmonella* in veal carcasses is 3.32% (95.00% Confidence Interval: 1.19% and 5.46%)
- The estimated national prevalence of *E. coli* O157:H7 in veal carcasses is 0.50% (95.00% Confidence Interval: 0.00% and 1.33%)
- The estimated national prevalence of non-O157 STEC in veal carcasses is 8.54% (95.00% Confidence Interval: 5.20% and 11.87%)

INTRODUCTION

The Food Safety and Inspection Service (FSIS) within 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. Moreover, 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 bacterial load (quantitative levels) of pathogens and indicator bacteria in raw products. Baselines are statistically designed microbiological surveys to assess and test food commodities for foodborne pathogens and bacteria that indicate process control. The survey measures industry as a whole by weighting sampling across strata according to the relative production volume of eligible establishments. The data generated by the baseline surveys are used to determine levels of pathogen and indicator bacteria in a particular commodity, establish microbiological industry criteria/standards, provide data for risk assessments, assess microbiological production parameters, and assess seasonal and regional variability. As such, baseline surveys are essential components for policy development and public health goals. The underlying principle of baselines is to present factual results as reflected by the data collected.

FSIS conducted the Beef Veal Carcass Baseline Survey (BVCBS) from August 2014 to December 2015. A 90-day training period preceded the survey (shakedown) for the field and laboratory personnel to prepare for the baseline. FSIS used the askFSIS system so that 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.

OBJECTIVES

Objective 1 - Estimate percent positive, prevalence, and bacterial load (quantitative level) of pathogenic organisms, including *Salmonella, Escherichia coli* O157:H7 and non-O157 Shiga-toxin producing *E. coli* (non-O157 STEC) on beef and veal carcasses. Detect the presence and estimate the quantitative levels of indicator organisms, including generic *E. coli*, Aerobic Count (AC), *Enterobacteriaceae* and total coliforms. In addition, obtain serotyping data for *Salmonella* isolates. **Objective 1 will be addressed in this report.**

Objective 2 - Obtain data for use in microbiological risk assessments to guide the development of Agency programs and guidance for industry related to process control.

Objective 3 - Perform post-hoc statistical analyses of the microbiological data when appropriate to explore the following additional issues:

1. Compare prevalence and bacterial counts between pathogenic and indicator organisms to determine relationships and associations; and,

2. Assess the effects of various factors on the microbiological profile (e.g., geographic region, seasonality, inspection system, plant size, and specific antimicrobial interventions).

Objectives 2 and 3 will be addressed in future reports and analyses.

METHODS

During this BVCBS, FSIS implemented the following specifications:

1. Sample beef and veal carcasses in federally inspected establishments at post-hide-removal and at pre-chill, before the carcass is further processed. These two points were selected because they encompass the processing of the entire carcass; pre-chill is the point of production before the carcass is broken down and is closest to the consumer within the establishment. Additionally at the point of pre-chill sampling, all antimicrobial interventions that are applied to the carcass are completed.

2. At the time of sample collection, FSIS inspectors also collected information related to the production conditions of the carcass from where the sample was collected; for example interventions applied establishment procedures, etc.

PROGRAM DESIGN

Establishments Included in the Sampling Frame[2]

In preparation for the survey, FSIS used data contained within the computerized database, the Public Health Information System (PHIS) to calculate production volume of establishments manufacturing beef and veal carcasses. FSIS identified 672 establishments that produced beef or veal. These establishments were potentially eligible for the baseline survey and represented all beef/veal produced in the United States under Federal Inspection. Due to limited resources, it was not feasible to sample all establishments; in fact, many establishments produced intermittently and in small quantities, making it difficult to reliably collect samples from these establishments. This led to the need to establish a cut-off production level that allows resource (sampling) allocation to establishments with stable production and substantial production volume. Minimum slaughter production levels for eligibility were established at 1,000 head/year for cattle and 10,000 head/year for veal calves. FSIS removed establishments from the sampling frame that did not meet these requirements to a total of 179 establishments producing beef and 12 establishments producing veal. These sampling frames for beef and veal are good representative samples of industry.

Sample Design and Collection

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 and inference is drawn on the population's parameters; non-sampling errors may be attributed to many sources inherent in the collection of samples, laboratory analysis, and data processing. FSIS considered these types of errors in determining the total sample size and the specific number of samples to be collected from each establishment to provide estimation of prevalence with an acceptable precision.

The beef survey incorporated a multi-stage cluster design that included sampling each establishment through the survey's duration. Multi-stage cluster or stratification allows for grouping establishments of similar size and calculating parameters in each stratum. By grouping the strata the parameters are recalculated for the entire population in a way that increases the accuracy of the estimation. Beef carcasses were selected at frequencies defined by each production volume categories and assigned a number of sampling events accordingly; a sampling event is the collection of 2 samples - 1 at post-hide-removal and 1 at pre-chill on the same carcass. After assessing the beef production volume, FSIS used the following volume categories for sampling frequency, sampling weight and calculation of prevalence:

Production Volume Category 1 consisted of high volume establishments that slaughter more than 1,000,000 head/year. This stratum contained plants that produced 58.00% of the total beef carcasses in the sampling frame. The target frequency collection for this stratum was 2 sampling events per month per establishment.

Production Volume Category 2 consisted of medium volume establishments that slaughter less than 1,000,000 head/year but more than 100,000 head/year. This stratum contained establishments that produced 37.00% of the total beef carcasses in the sampling frame. The target frequency collection for this stratum was 4 sampling events every 3 months per establishment.

[2]Statistically, a sampling frame is the source (establishments) from which a sample is drawn. It is a list of all the elements (establishments) within a population that are eligible for sampling. These establishments are eligible but not necessarily sampled during the survey.

Production Volume Category 3 consisted of small volume establishments that slaughter less than 100,000 head/year. This stratum contained establishments that produced 5.00% of the total beef carcasses in the sampling frame. The target frequency collection for this stratum was 1 sampling event every two months per establishment.

For the veal sampling frame design, samples were drawn from plants slaughtering over 10,000 head/year and accounts for 93.00% of all veal production regulated by FSIS. Because the group of veal producing establishments was small, there was no need for stratification in the veal sampling frame. These establishments were sampled at a rate of two sampling events per month per establishment.

Actual Survey Collection

Sample collection for the BVCBS was originally scheduled for a consecutive 12 month period beginning August 2014 and was extended to December 2015. FSIS monitored the overall rate of sample collection and other parameters during the survey. Three establishments scheduled to be sampled that ceased production were replaced with equivalent establishments. FSIS accounted for samples that were not collected according to schedule as well as samples that were discarded and therefore not analyzed. Establishments that had annual productions that crossed the strata boundaries were relocated to their corresponding stratum. During the survey, because the rate of sample collection was below that required for a statistical valid survey, the survey was extended until approximately 3,200 samples were collected. At the end of the survey FSIS created a file with the laboratory results that was used for all calculations presented in this document.

A summary of the final paired beef samples by strata is presented in Table A.

Table A. Beef summary survey by strata

Strata	Establishments	Samples	Percent of Sampled Production	Allocation of Samples by Strata
1	13	322	70.50%	23.50%
2	30	446	27.86%	32.60%
3	96	600	1.64%	43.90%
Total	139	1,368	100.00%	100.00%

For veal, a single stratum was formed with 12 establishments from which 274 samples were collected at post-hide-removal and 274 at pre-chill. Averages of 23 paired samples per establishment were collected during the survey.

Sampling Location within Establishments

Samples were collected at two locations:

1 Post-hide-removal - this is immediately after de-hiding, before evisceration, and if possible before the establishment applies any antimicrobial or hot water interventions to the exposed carcass surface.

2 Pre-chill – the spot which is at least 1 - 5 minutes after the establishment applies its last intervention on the slaughter floor and no later than one hour inside the hot box, preferably as soon as possible after the 1 to 5 minute wait time, before the carcass enters the cooler. If additional interventions are applied to the carcass during chilling, the sample was collected before the application of those interventions.

Sample Collection and Description

Samples were aseptically collected by FSIS inspectors following the procedures described in FSIS Notice 36-14 issued on July 25, 2014 and posted on the FSIS website. Additionally, a demonstration of sampling collection was presented in a YouTube video.

The carcass samples consisted of sponges collected at post-hide-removal and pre-chill by swabbing an area of 8,000 cm^2 for beef; one sponge swab at the posterior area of the carcass covering approximately 4,000 cm^2 and another sponge swab at the anterior area of the carcass covering about 4,000 cm^2; that is, two sponges covering a surface of 8,000cm^2 of the beef carcass. For veal, the surface swabbed was 4,000 cm^2; one sponge swab at the posterior area of the carcass covering approximately 2,000 cm^2 and another sponge swab at the anterior area of the carcass covering about 2,000 cm^2; that is, two sponges representing 4,000cm^2 of the veal carcass.

Two sponges per carcass were collected for each sampling location (post-hide removal and pre-chill); one sponge from the posterior area and the second sponge from the anterior area. Because each carcass was sampled at two locations, four sponges were sent to the lab (i.e., one anterior at post-hide-removal, one posterior at post-hide-removal, one anterior at pre-chill, and one posterior at pre-chill). Samples shipped to the lab also included corresponding information on the samples (sample form); if the sample form or any sponge was missing the sample was discarded. Specific instructions to personnel as well as sample collection procedures are detailed in FSIS Notice 36-14.

The samples were shipped to the FSIS laboratory by an overnight delivery service on the same day they were collected or the next day if the sample was collected late in the day. 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^{o}C to 15^{o}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 three pathogens – *Salmonella, E.coli* O157:H7 and non-O157 STEC (Shiga toxin-producing *E. coli* that have one of these following O antigens: O26, O45, O103, O111, O121, or O145 and the virulence genes *stx* and *eae*) and for indicator organisms - generic *E. coli*, total coliforms, aerobic count, and *Enterobacteriaceae*. In addition, *Salmonella* isolates were serotyped.

SAMPLE ANALYSIS METHODS

Sample Preparation

Two sponges (posterior and anterior) for each sample location (post-hide removal and pre-chill) were composited and brought to a final volume of 50 mL buffered peptone water (BPW). A reserve sample for conducting *Salmonella*, *E. coli* O157:H7, and non-O157 STEC Most Probable Number (MPN) analyses was prepared by adding 5mL of 50.00% glycerol to 10mL of the composited sponge sample. The reserve sample was stored frozen until MPN analyses were conducted on confirmed positive samples.

Indicator Bacteria

To analyze the samples for the indicator bacteria, a 1:40 dilution was obtained by adding 0.1 mL sponge diluent to 3.9 mL of sterile deionized (DI) water in a reagent vial. A 1:40,000 dilution was also obtained by first making a 1:10 dilution, adding 0.1 mL of the dilution to 9.9 mL of 0.85% saline and adding 0.1 mL of that dilution to 3.9 mL of sterile DI water in a reagent vial. Dilutions were homogenized for approximately three seconds before being analyzed via TEMPO®(2). The level for each indicator aerobic count (AC), *Enterobacteriaceae*, coliforms, and generic *E. coli* was estimated using the MPN procedure. The value within the range of the count was reported as the result; in the event of an overlap of countable results, the average was calculated and reported. In addition, to convert ranges to continuous numbers, ranges “less than” the limit of detection (LOD) were divided by two and the outcome used as a result for MPN; for results “greater than” a 10.00% was added and the outcome used as the continuous result.

Salmonella

The sponge samples were analyzed for the presence of *Salmonella* using the carcass sponge instructions listed in (2) (3) . Samples were enriched with mTSB and incubated at 42°C for 15-24 hrs. An aliquot of the enriched sample was screened for *Salmonella* using the DuPont Qualicon BAX system. The level of *Salmonella* in the confirmed positive samples was estimated using the MPN procedure. The presence of *Salmonella* from a positive screen test was culture-confirmed by FSIS MLG 4.08. From the reserve sample, five dilutions (three tubes per dilution) were obtained: 10 mL, 1 mL, 0.1 mL, 0.01 mL, and 0.001 mL. 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 reported as "MPN/100cm^2". 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.

STEC

***Escherichia coli* O157:H7**

The sponge samples were analyzed for the presence of *E. coli* O157:H7 using the carcass sponge instructions listed in (4) (5). Sponge samples were enriched with mTSB and incubated at 42°C for 15-24 hrs. An aliquot of the enriched sample was screened for *E. coli* O157:H7 using the DuPont Qualicon BAX system. The presence of *E. coli* O157:H7 from a positive screen test was culture confirmed by FSIS MLG 5.09 (January 15, 2015). The level of *E. coli* O157:H7 in the confirmed positive samples was estimated using the MPN procedure. From the reserve sample, five dilutions (three tubes per dilution) were obtained: 10 mL, 1 mL, 0.1 mL, 0.01 mL, and 0.001 mL. The pattern of positives and negative results among these individual qualitative tests was used to estimate the levels of *E. coli* O157:H7 and the results were expressed as "MPN/100cm^2".

Non-O157 STEC

The sponge samples were analyzed for the presence of non-O157 STEC using the carcass sponge instructions listed in (6) (7). Sponge samples were enriched with mTSB and incubated at 42°C for 15-24 hrs. An aliquot of the enriched sample was screened for *stx*/*eae* genes and STEC Panel 1 and Panel 2 using the DuPont Qualicon BAX system. The presence of non-O157 STEC was culture confirmed by the FSIS MLG method. The level of non-O157 STEC for the O group(s) that confirmed positive was estimated using the MPN procedure (8). From the reserve sample, five dilutions (three tubes per dilution) were obtained: 10 mL; 1 mL; 0.1 mL; 0.01 mL; and, 0.001 mL. The pattern of positive and negative results among these individual qualitative tests was used to estimate the levels of *E. coli* non-O157 STEC and the results were expressed as "MPN/100cm^2".

DATA ANALYSIS METHODS

General Overview

The data analysis of this survey was mainly conducted using the statistical software JMP v. 11 (9). This software was also used to create the tables, figures, maps, and statistical tests analyses presented in this report. In addition JMP v. 11 software was used in the file merges new variables formulation, conditional statements, variable conversions, summaries, etc. An important outcome of this report is the calculation of the estimates of national prevalence of *Salmonella, E. coli* O157:H7 and non-O157 STEC in beef and veal carcasses. The national prevalence provides a national average of expected values for these pathogens on beef and veal carcasses at specific points in the slaughter process. Sampling for the survey was designed to be representative of all plants producing beef and veal carcasses under FSIS inspection.

For beef producing establishments the design approach used class or "strata" by production volume; the strata were defined as large, medium, and small establishments. 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 establishments (which are the primary sampling unit (PSU) or place were the sample is taken) were weighted by assigning each corresponding establishment's fractional contribution of the total national production. Once weight was assign to each establishment the individual sample weight was calculated by taking shares in direct proportion to the number of samples taken from the establishment weight. After these considerations, the specialized statistical software WesVar v 5.1 (10) was used to calculate the estimate of national prevalence and its uncertainty. Details of file development, sample

weight calculation (prevalence), and software processing are presented in the appendix (Statistical Analysis Plan (SAP)).

Given the small amount of veal producing establishments all veal producing establishments were included in a single group (no stratification). Establishment weight and individual sample weight was computed without the need for stratification considerations (described in the SAP). Calculation of national prevalence for veal was done using JMP software.

RESULTS / DISCUSSIONS

The BVCBS analyzed 2,736 beef samples collected, 1,368 at post-hide-removal and 1,368 at pre-chill from 139 establishments with an average of 10 samples per establishment. For veal, 548 samples were collected, 274 at post-hide-removal and 274 at pre-chill from 12 establishments with an average of 23 samples per establishment collected and analyzed. The post-hide-removal and pre-chill samples were paired by collecting the samples from different sides of the same carcass (i.e. the post-hide-removal sample was taken on one half of the carcass and the pre-chill sample was taken on the other half of the same carcass).

Microbiological Results

The analytical results of the survey are summarized in a series of tables for samples at post-hide removal and pre-chill. Post-hide removal is a point at the beginning of the fabrication process where the carcass is de-hided and initial interventions are applied. Pre-chill is the point in the establishment closer to the consumer before the carcass is further processed and the spot which is at least 1 - 5 minutes after the establishment applies its last intervention on the slaughter floor and no later than one hour inside the hot box, preferably as soon as possible after the 1 to 5 minute wait time, when the carcass enters the cooler. If additional interventions are applied to the carcass during chilling, the sample was collected before the application of those interventions. **Table 1** and **Table 2** presents a compilation of the test results of all quantified samples for beef carcasses at post-hide removal and pre-chill and combines all the results for pathogens and indicator organisms. **Table 3** and **Table 4** present the same results for veal at post-hide removal and pre-chill.

For beef carcasses at post-hide removal, the percent positive for *Salmonella* was 27.12%, 1.83% for *E. coli* O157:H7 and 6.14% for non-O157 STEC. For beef carcasses at pre-chill the percent positive for *Salmonella* was 3.36%, 0.66% for *E. coli* O157:H7 and 0.73% for non-O157 STEC.

For veal carcasses at post-hide removal, the percent positive for *Salmonella* was 12.04%, 0.73% for *E.coli* O157:H7, and 23.72% for non-O157 STEC. For veal carcasses at pre-chill, the percent positive for *Salmonella* was 1.82%, 0.73% for *E.coli* O157:H7, and 9.85% for non-O157 STEC. Tests of proportions were done to compare statistically beef and veal results for pre-chill samples. *Salmonella* on beef at 3.36% vs veal at 1.82% shows no significant difference (p-value = 0.18) and *E. coli* O157:H7 on beef at 0.66% vs veal at 0.73% also show no significant difference (p-value = 0.89). However, 0.73% of non-O157 STEC found on beef carcasses vs 9.85% on veal carcasses is significantly different (p-value < 0.001). The results indicate that veal carcasses have a higher likelihood of contamination with non-O157 STEC when compared to beef carcasses at pre-chill.

A comparison of indicators using the geometric mean for beef vs veal at post-hide removal is presented in **Table 5** and the same comparison for pre-chill was done and results are reported on **Table 6**. The geometric mean was selected for the comparison because it is the most stable measure of central tendency for indicator distributions. The comparison, using a non-parametric Wilcoxon/Kruskal-Wallis test, shows that all four indicators are significantly higher on veal. This may indicate that veal carcasses are prone to carry more indicator microorganisms. It also may indicate that sanitary interventions for veal carcasses are not comparable with beef carcasses.

***Salmonella* Serotyping**
For each *Salmonella* positive sample, one colony was picked and the serotype was determined.

The *Salmonella* serotypes isolated most often on beef carcasses at post-hide-removal were Montevideo (21.56%), Anatum (15.90%) and Cerro (10.78%). There were 46 different serotypes in 371 positive samples and **Table 7** shows the frequencies and percentages calculated for the top 10 most frequent *Salmonella* serotypes detected in post-hide-removal samples.

The *Salmonella* serotypes isolated most often on beef carcasses at pre-chill were Montevideo (17.39%), Muenchen (10.87%), Agona (8.70%) and I 4,[5],12:i:- (8.70%). There were 20 different serotypes in 46 positive samples and **Table 8** shows the frequencies and percentages calculated for the top 10 most frequent *Salmonella* serotypes isolates identified in pre-chill samples.

The *Salmonella* serotypes isolated most often on veal carcasses at post-hide-removal were Cerro (21.21%), Montevideo (12.12%) and Newport (12.12%). A total of 14 different serotypes were isolated from 33 positive samples. In post-hide-removal samples, **Table 9** shows the frequencies and percentages calculated for the top 10 most frequent *Salmonella* serotypes isolated.

Only three different *Salmonella* serotypes were isolated from veal at pre-chill in five positive samples; Newport (40.00%), Typhimurium (40.00%) and Anatum (20.00%). **Table 10** shows the frequency and percentages calculated for these serotypes isolated from pre-chill samples.

Calculation of Prevalence
FSIS calculated the prevalence or weighted average in relation to production volume for pathogens for both beef and veal at pre-chill. Because prevalence estimates are weighted calculations in relation to production volume and percent positive is calculated from the number of positives found in the sample, national prevalence estimates may be slightly different from the reported percent positive. **Figure 1** shows the WesVar software output window for prevalence of *Salmonella, E. coli* O157:H7 and non-O157 STEC at pre-chill in beef carcasses. In summary the results of prevalence are:

- The estimated national prevalence of *Salmonella* in beef carcasses is 0.72% (95.00% Confidence Interval: 0.22% and 1.21%)
- The estimated national prevalence of *E. coli* O157:H7 in beef carcasses is 0.06% (95.00% Confidence Interval: 0.00% and 0.15%)
- The estimated national prevalence of non-O157 STEC in beef carcasses is 0.21% (95.00% Confidence Interval: 0.00% and 0.51%)

Usually, prevalence calculations are close to the percent positive; however, compared to the reported percent positive, the prevalence estimates were significantly lower, i.e., *Salmonella* percent positive was 3.36% compared to a prevalence of 0.72%; *E. coli* O157:H7 percent positive was 0.66% compared to a prevalence of 0.06%; and non-O157 STEC percent positive was 0.73% compared to a prevalence of 0.21%. All reported percent positive values were above the upper 95.00% confidence limit calculated for corresponding prevalence. This unexpected reduction of prevalence indicates that a causal effect is present and further review is necessary to determine the cause of the noteworthy differences. The analyst assembled two tables with the positives results by strata, one for post-hide removal and another for pre-chill. **Table 11** shows the distribution by strata at post-hide removal for reference.

Table 12 shows that at pre-chill the overwhelming majority of positives are in stratum 3, which includes small establishments (89.00% for *Salmonella* positives, 100.00% for *E. coli* O157:H7 positives and 90.00% for non-O157 STEC positives). Small establishments provide a smaller proportion when weighted since its production volume is far less in comparison with larger establishments. Because the majority of positive samples are in the third strata, the calculations of weighted averages, and thus the national prevalence estimates, diminish accordingly. This explains why the national prevalence estimate is much lower than the percent positive. This finding indicates that products from small beef producing establishments are more likely to be contaminated with the pathogens analyzed in this study than are larger beef producing establishments.

The calculation of prevalence for veal used the simple weight of the establishments because the sample design for veal was not stratified. Results for prevalence and its uncertainty were estimated using the statistical software JMP.

- The estimated national prevalence of *Salmonella* in veal carcasses is 3.32% (95.00% Confidence Interval: 1.19% and 5.46%)
- The estimated national prevalence of *E. coli* O157:H7 in veal carcasses is 0.50% (95.00% Confidence Interval: 0.00% and 1.33%)
- The estimated national prevalence of non-O157 STEC in veal carcasses is 8.54% (95.00% Confidence Interval: 5.20% and 11.87%)

Prevalence results are slightly different from the reported percent positive for pathogens of interest on veal and all reported percent positive values are within the 95.00% confidence interval of the estimation of national prevalence (*Salmonella* percent positive 1.82% vs national prevalence 3.32%; *E. coli* O157:H7 percent positive 0.73% vs national prevalence 0.49%, and non-O157 STEC percent positive 9.85% vs national prevalence 8.53%). These moderate differences are produced because of the introduction of production volume weighting necessary to compensate for the survey's design bias.

Comparison of Pathogens and Indicators within Species and within Animal Class

FSIS was interested in analyzing the differences between beef and veal as well as differences within an animal class. **Table 13** shows the differences between beef and veal by pathogens at post-hide-removal. Although FSIS applies the same policies to both beef and veal, the results show that beef carcasses (27.12%) have significantly higher (p-value < 0.0001) *Salmonella* percent positive than veal carcasses (12.04%). For *E. coli* O157:H7, there was no significant difference, 1.83% for beef vs 0.73% for veal (p-value = 0.19). Moreover, for non-O157 STEC, veal at 23.72% positive is significantly higher (p-value < 0.0001) than beef with 6.14% positive. Similarly, at pre-chill, **Table 14** shows that *Salmonella* percent positive (3.36% vs 1.82%) and *E. coli* O157:H7 (0.65% vs 0.73%) are not significantly different between beef and veal. However, non-O157 STEC on veal are significantly higher (p-value < 0.0001) than on beef (0.73% for beef vs 9.85% for veal). These results may indicate that non-O157 STEC are more likely to contaminate veal (non-ruminating) and it may be more difficult to eliminate the pathogen, due to the thickness of the hide, environmental, physiological, or other factors.

As stated in the "FSIS Compliance Guidelines for Minimizing the Risk of Shiga Toxin-Producing *Escherichia coli* (STEC) and *Salmonella* in Beef (including Veal) Slaughter Operations" (11), establishments apply a number of different practices during the slaughter process to reduce or eliminate microorganisms on carcasses. These processes can include various sanitary dressing practices and the application of antimicrobial interventions on the carcass.

Table 15 shows the reductions of bacteria from post-hide-removal to pre-chill by pathogen. Except for *E. coli* O157:H7 in veal, there was a significant reduction of pathogens between the two sampling points. The non-significant result for *E. coli* O157:H7 may be explained by the low number of positive results observed in veal, two at post-hide-removal and two at pre-chill.

The reduction in the level of indicator bacteria between the two sampling points also suggests that common practices in the establishment reduce the potential contamination. **Table 16** and **Table 17** present the levels of indicator bacteria at post-hide-removal and at pre-chill for beef and veal respectively; all results are in MPN/100cm^2. The data show a significant decrease (p-value < 0.001) for all indicators examined for both beef and veal. To describe the distributions at post-hide-removal and pre-chill, the tables present three parameters of central tendency (mean, median and geometric mean) for each indicator and corresponding log value (log is assumed to be logarithm base 10 i.e., $\log_{10}$); an additional column calculates the reduction of indicators including the log reduction.

A final column indicates whether the reduction from post-hide-removal to pre-chill is statistically significant. A preliminary goodness-of-fit test shows that distributions of indicators are not normal (Shapiro-Wilk W test with p-value <0.001). Because the distributions of indicators are not normal and they are highly skewed to the right (many results are close to LOD and a few results are much higher) a non-parametric test was used to

determine if there was statistical difference from post-hide-removal to pre-chill. All indicators were found significant with lower concentration levels at pre-chill for both beef and veal. The p-values in Table 16 and Table 17 result from the application of non-parametric Wilcoxon / Kruskal-Wallis test (Rank Sums).

FSIS reviewed the pathogen percent positive among animal classes. For beef, **Table 18** shows the pathogen percent positive distributed by animal class at post-hide-removal. Cows and bulls, as well as, steers and heifers were combined into a single group Cow/Bull and Steer/Heifer. A test of proportions displays that *Salmonella* on dairy cows has a significantly higher (p-value = 0.02) percent positive when compared to Steer/Heifer but not when compared to Cow/Bull (p-value = 0.25); *E. coli* O157:H7 and non-O157 STEC present no statistical significant differences at post-hide-removal. These results may indicate that dairy cows have a predisposition to carry a higher percent positive of *Salmonella* in relation to Steer/Heifer when coming into the establishments. **Table 19** shows a similar situation at pre-chill with dairy cows; with significantly higher percent of *Salmonella* when compared to Steer/Heifer and no significant difference for *E. coli* O157:H7 and non-O157 STEC. Similar *Salmonella* proportional reductions in these two tables may suggest that the reduction of *Salmonella* is independent of the animal class and is a function of the quantity of pathogen levels that existed at the beginning of the process.

For veal, only two animal classes were compared: bob-veal and formula-feed veal; the other two animal classes, heavy calf and non-formula-fed veal, have a sample size too small for meaningful comparison. According to **Table 20** at post-hide-removal, *Salmonella* is significantly higher for bob-veal (29.90%) when compared to formula-feed veal (2.31%). The significant percent positive *Salmonella* difference between bob veal and formula-fed veal suggests that *Salmonella* is more prevalent on incoming bob veal. *E.coli* O157:H7 and non-O157 STEC were not significantly different between these animal classes. The same pattern is observed at pre-chill in **Table 21**. *Salmonella* on bob-veal is significantly higher (5.15% vs 0.00%) with p-value = 0.02 while *E. coli* O157:H7 and non-O157 STEC are not significantly different. The similar *Salmonella* high percent positive observed in both Tables 20 and 21 may suggest that the reduction of *Salmonella* is independent of the animal class and is a function of the levels of pathogen that existed at the beginning of the slaughter process.

Non-O157 STEC on Veal

FSIS noted the predominance of non-O157 STEC on veal carcasses in relation to beef carcasses; the percent positive at post-hide removal in beef was 6.14% vs 23.72% in veal and at pre-chill, beef was 0.73% vs veal at 9.85%. **Table 22** and **Table 23** provide details on the distribution of non-O157 STEC on veal at post-hide-removal and pre-chill, respectively. These Tables demonstrate that O103 and O111 are the most common non-O157 STEC serogroups associated with veal carcasses. At post-hide-removal they contribute to 90.77% (59/65) of all positives and 88.89% (24/27) at pre-chill.

Source of Pathogens Positives per Establishments

In relation to pathogens at pre-chill, there are establishments that operate in optimal conditions while others lack efficiency in eliminating pathogens throughout the process. It is important to point out establishments that heavily contribute to the overall number of positives. From the 139 beef establishments sampled in the survey **Table 24** captures the top 10 establishments with testing positive for *Salmonella* at post-hide removal and **Table 25** captures the top 10 establishments with testing positive for *Salmonella* at pre-chill. For pre-chill these 10 establishments (10 out of 139 or 7.20%) contribute almost half (22 out of 46 or 47.80%) of all *Salmonella* positives recorded at pre-chill. Nine of these establishments are in stratum 3 and one is in stratum 2.

For veal, data from all 12 establishments that were sampled are presented in **Table 26** for post-hide removal and on **Table 27** for pre-chill. At pre-chill, Non-O157 STEC are the primary pathogens identified in veal and one single establishment contributed 33.00% (9/27) of all non-O157 STEC positives recorded at pre-chill and only 2 establishments had no non-O157 STEC positive samples.

Pathogen Trends during the Survey

The BVCBS lasted 17 months and FSIS wanted to know if there was any seasonal trend during that time. **Figure 2** presents the trend for *Salmonella* at post-hide-removal for beef. Post-hide-removal is closest to the entry

point of the carcasses into the establishment. The Figure shows a smooth curve (with Lambda = 0.02)[3] of the variation of *Salmonella* percent positive during the survey (blue curve). The *Salmonella* average of 27.10% for beef carcasses at post-hide-removal (red line), observed during the survey is added for reference. The *Salmonella* percent positive went as high as 40.00% around August-September 2014 and went down to about 17.00% in January 2015. By June 2015 it rose again above the average. The Figure suggests that contamination with *Salmonella* is more likely to occur in warmer months from June to October.

In veal the most prevalent pathogens identified are the non-O157 STEC and **Figure 3** shows the smooth curve (with Lambda = 0.02) of the variation of non-O157 STEC percent positive during the survey (blue curve). The non-O157 STEC average of 23.70% for veal carcasses at post-hide-removal (red line), observed during the survey is added for reference. The smooth curve shows the highest percent positive, above 35.00%, around January and April and very low percent positive around July-September 2015. Sample size distributed by month is small and any suggestion drawn from this curve is not robust.

Reduction of Microorganism via Interventions

FSIS sample collectors were asked to report the types of interventions that were applied to hides/carcasses prior to the sample collection site. The survey found that at post-hide-removal, interventions ranged from no intervention at all to several interventions applied to the same carcass prior to sampling the carcass. The summary presented on **Table 28** shows eleven different interventions applied to beef carcasses prior to the post-hide-removal sampling point. The most frequent intervention at 26.56% is the application of cold water washing and the least frequent is acetic acid hide-on carcass wash, representing 0.10% of the applications. Because several beef carcasses received multiple interventions, **Figure 4** provides the number of beef carcasses that received 1, 2 and up to 5 interventions each; included are the number of beef carcasses that received no interventions at all. The Figure shows the number of carcasses and the percentage they represent, i.e., 414 carcasses or 30.20% received only one intervention while 38 carcasses or 3.00% received 3 interventions.

The number of beef carcasses that received no intervention prior to the post-hide-removal sampling point is noticeable, 725 carcasses or 53.00% of the samples taken. This observation presents an opportunity for an even partitioning of the samples to investigate the effectiveness of treatments by dividing the samples collected at post-hide-removal sampling point into two groups, one group treated (receiving one or more interventions) and the other untreated. Once divided in treated carcasses and untreated carcasses, FSIS explored the percent positive for each group. **Table 29** compares treated vs untreated beef carcasses for pathogens at post-hide-removal. Proportion tests at 95% confidence were calculated and for *Salmonella* there was no significant difference (p-value = 0.30) between the treated group at 25.80% vs 28.30% for the untreated beef carcasses; for *E. coli* O157:H7 there also was no significant difference (p-value = 0.18) between treated beef carcasses at 2.30% vs untreated beef carcasses at 1.40%. The same was found for non-O157 STEC: treated beef carcasses 5.00% vs untreated beef carcasses at 7.20% (p-value = 0.09). Although these statistical results (p-value = 0.05) may suggest that treatment at post-hide-removal may not contribute in a substantial way to the reduction of pathogens in beef carcasses, there are evidences that interventions are a valuable way to reduce pathogens.

In addition, sanitary removal of the hide (which is the first step) is essential for proper dressing to minimize gross cross contamination. It is worth noting that 55 (8.70%) of untreated samples came from stratum one, 187 (29.40%) of untreated samples came from stratum two and the majority, 393 (61.90%) of untreated samples came from stratum three or small establishments. Previous analysis indicates that the majority of pathogen positive results came from the third stratum and this abundance of positive results in the third stratum may be linked to the lack of carcass treatment in that stratum. Additionally, FSIS further evaluated the comparison of treated vs untreated carcasses by bacterial load using MPN results; however the majority of results are below the LOD. The distributions present huge standard deviations, and once the samples are

[3] A smooth curve or smoother function makes a pointy curve smooth by attenuating the sharp changes from one value to the other. The value of lambda (λ) measures the smoothness of the curve; the curve gests smoother with higher values of lambda and less smooth when lambda approaches 0.

divided into treated and untreated, the sample size is reduced to fewer samples. All these factors make the MPN comparison not informative.

FSIS investigated if treated vs untreated at the post-hide-removal sampling point had an impact at pre-chill. **Table 30** contains the number of pathogen positives and the percent positive for beef carcasses at pre-chill. Tests of proportions show no difference for *E. coli* O157:H7 (p-value = 0.18) and no difference for non-O157 STEC (p-value = 0.98). However, for *Salmonella* there was a significant difference at pre-chill, 4.40% for untreated carcasses vs 2.20% for treated carcasses (p-value = 0.04). This result suggests that treatment prior to the post-hide-removal sampling point helps to reduce *Salmonella* levels in beef carcasses at pre-chill.

Distribution of interventions on beef carcasses prior to the pre-chill sampling point also is of interest . **Table 31** lists the interventions that were reported. The interventions most frequently reported were trimming (20.08%) and hot water carcass wash (15.57%); the least frequent was the hand-held application of hypobromous acid (0.10%) of all applications. **Figure 5** enumerates the number of beef carcasses that received no interventions to those that received up to 10 interventions on the same carcass. Only 20 (0.50%) beef carcasses did not receive any intervention prior to the pre-chill sampling point and 923 (67.00%) beef carcasses received 1 to 3 interventions.

Table 32 shows the distribution of interventions applied to veal prior to the post-hide-removal sampling point. The most common intervention was hot water washing (26.32%) and cold water washing (16.45%) was the second most commonly reported intervention. Fifty-seven (21.00%) of the sampled veal carcasses did not receive any intervention and 136 (50.00%) of the 274 veal carcasses received a single intervention as seen in **Figure 6**.

Table 33 presents the listing of interventions applied to veal carcasses prior to the pre-chill sampling point; the most common was trimming 145 (20.80%), followed by hot water carcass wash 106 (15.21%). **Figure 7** shows the number of interventions applied to a single carcass. Twelve (4.00%) veal carcasses did not have interventions and 189 (70.00%) veal carcasses received 1 to 3 interventions prior to the pre-chill sampling point.

CONCLUSIONS

This survey on beef and veal carcasses was conducted by FSIS and designed to determine presence and levels of pathogens on the entire beef and veal carcasses before further processing in federally inspected plants. In response to Objective 1 of the study, FSIS determined the national prevalence of *Salmonella*, *E. coli* O157:H7 and non-O157 STEC which are essential to develop industry standards in the future. Additionally, FSIS presented in this report information on *Salmonella* serotypes, comparison of pathogens and indicators within species and within animal class, a review of non-O157 STEC on veal, investigation of pathogen positives per establishment, pathogen trends during the survey, and a look at reduction of bacterial levels via interventions. All these results may help the beef and veal industry to assess the state of carcass contamination and to make informed decisions to improve the processing procedures or environment. The baseline results will also help FSIS to assess and regulate industry objectively. Data and information collected during the survey will serve as the foundation for satisfying Objectives 2 and 3 of this study and will inform further analyses to establish risk assessments, industry process control criteria and improve policy decisions and general knowledge of the beef and veal industry. FSIS acknowledges all the individuals that participated in this complex survey and recognizes that without the active participation of inspectors, laboratory personnel, and headquarters personnel, this project could not have been accomplished.

TABLES AND FIGURES
Data Source – FSIS databases LIMS/PHIS, February 2016

Table 1. Summary for Quantified Beef Samples at Post-hide Removal by Microorganism in the 2014-2015 Beef Veal Carcass Baseline Survey

Microorganisms Indicator Organism [1]	Sample Collected at	Number of Samples Tested	Number of Samples Quantifiable [2]	Percent Positive	Quantitative Levels [3] Mean (Data units)	Mean Std Error	Geometric Mean	Geo Mean 95% CI	Log 10 of the Geo Mean
Aerobic Count	Post-hide Removal	1,348	1,341	99.48%	340,973	66,960	8,946.0	(7,873 – 10,163)	3.95
Enterobacteriaceae	Post-hide Removal	1,366	1,020	74.67%	17,724	16,929	30.0	(26.8 – 33.6)	1.47
Total Coliforms	Post-hide Removal	1,366	922	67.50%	2,627	2,154	19.7	(17.7 – 21.9)	1.29
Generic Escherichia coli	Post-hide Removal	1,365	1,034	75.75%	6,472	2,531	40.8	(35.9 – 46.5)	1.61
Pathogenic Organism [4] [5]									
Salmonella	Post-hide Removal	1,368	371	27.12%	1.53	1.11	0.20	(0.18 – 0.22)	-0.71
E. coli O157:H7	Post-hide Removal	1,368	25	1.83%	0.20	0.05	0.16	(0.13 – 0.20)	-0.79
non-O157 STEC [6]	Post-hide Removal	1,368	84	6.14%	4.52	4.10	0.30	(0.24 - 0.37)	-0.52

(1) Units are MPN/100 cm^2 and limit of Detection (LOD) < 6.25 MPN/100 cm^2

(2) This is results above Limit of Detection (LOD); LOD < 6.25 MPN/100cm^2

(3) Calculations include estimations for results under LOD < 6.25 at LOD/2 = 3.12 MPN/ 100cm^2

(4) Calculations include results under LOD < 0.2815 and estimated at LOD/2 = 0.14

(5) Pathogens results are in MPN/100 cm2 with LOD < 0.2815 MPN/100cm2

(6) Non-O157 STEC are: O26, O45, O103, O111, O121, and O145. Table shows aggregate results.

Data Source – FSIS databases LIMS/PHIS, February 2016

Back→

Table 2. Summary for Quantified Beef Samples at Pre-Chill by Microorganism in the 2014-2015 Beef Veal Carcass Baseline Survey

Microorganisms Indicator Organism [1]	Sample Collected at	Number of Samples Tested	Number of Samples Quantifiable [2]	Percent Positive	Quantitative Levels [3] Mean (Data units)	Mean Std Error	Geometric Mean	Geo Mean 95% CI	Log 10 of the Geo Mean
Aerobic Count	Pre-Chill	1,359	1,094	80.50%	89,591	30,485	106.8	(90 – 127)	2.02
Enterobacteriaceae	Pre-Chill	1,366	306	22.40%	380	223	5.4	(5.0 – 5.8)	0.73
Total Coliforms	Pre-Chill	1,366	229	16.76%	25,528	22,528	4.8	(4.4 – 5.2)	0.68
Generic Escherichia coli	Pre-Chill	1,368	189	13.82%	452	244	4.4	(4.1 – 4.7)	0.64
Pathogenic Organism [4][5]									
Salmonella	Pre-Chill	1,368	46	3.36%	0.19	0.03	0.16	(0.14 – 0.19)	-0.78
E. coli O157:H7	Pre-Chill	1,368	9	0.66%	0.14	0.00	0.14	(0.14 – 0.14)	-0.85
non-O157 STEC [6]	Pre-Chill	1,368	10	0.73%	0.21	0.05	0.18	(0.12 - 0.26)	-0.74

(1) Units are MPN/100 cm^2 and limit of Detection (LOD) < 6.25 MPN/100 cm^2

(2) This is results above Limit of Detection (LOD); LOD < 6.25 MPN/100cm^2

(3) Calculations include estimations for results under LOD < 6.25 at LOD/2 = 3.12 MPN/ 100cm^2

(4) Calculations include results under LOD < 0.2815 and estimated at LOD/2 = 0.14

(5) Pathogens results are in MPN/100 cm2 with LOD < 0.2815 MPN/100cm2

(6) Non-O157 STEC are: O26, O45, O103, O111, O121, and O145. Table shows aggregate results.

Data Source – FSIS databases LIMS/PHIS, February 2016

Back→

Table 3. Summary for Quantified Veal Samples at Post-hide Removal by Microorganism in the 2014-2015 Beef Veal Carcass Baseline Survey

Microorganisms Indicator Organism [1]	Sample Collected at	Number of Samples Tested	Number of Samples Quantifiable [2]	Percent Positive	Quantitative Levels [3] Mean (Data units)	Mean Std Error	Geometric Mean	Geo Mean 95% CI	Log 10 of the Geo Mean
Aerobic Count	**Post-hide Removal**	272	271	99.63%	575,654	286,655	14,057.0	(10,405 – 18,990)	4.14
Enterobacteriaceae	**Post-hide Removal**	274	202	73.72%	5,705	1,988	95.0	(68.9 – 131.1)	1.98
Total Coliforms	**Post-hide Removal**	271	180	66.42%	22,939	19,854	61.3	(45.2 – 83.2)	1.79
Generic Escherichia coli	**Post-hide Removal**	274	193	70.44%	5,592	2,021	79.6	(58.1 – 109.0)	1.90
Pathogenic Organism [4] [5]									
Salmonella	**Post-hide Removal**	274	33	12.04%	0.37	0.05	0.32	(0.27 – 0.37)	-0.49
E. coli O157:H7	**Post-hide Removal**	274	2	0.73%	0.28	0.00	0.28	(0.28 – 0.28)	-0.55
Non-O157 STEC [6]	**Post-hide Removal**	274	65	23.72%	0.50	0.19	0.44	(0.35 - 0.56)	-0.35

(1) Units are MPN/100 cm^2 and limit of Detection (LOD) < 12.5 MPN/100 cm^2

(2) This is results above Limit of Detection (LOD); LOD < 12.5 MPN/100cm^2

(3) Calculations include estimations for results under LOD at LOD/2 = 6.25 MPN/ 100cm^2

(4) Calculations include results under LOD <0.5625 and estimated at LOD/2 = 0.28125 MPN/ 100cm^2

(5) Pathogens results are in MPN/100 cm2 with LOD < 0.5625 MPN/100cm2

(6) Non-O157 STEC are: O26, O45, O103, O111, O121, and O145. Table shows aggregate results.

Data Source – FSIS databases LIMS/PHIS, February 2016

Back→

Table 4. Summary for Quantified Veal Samples at Pre-Chill by Microorganism in the 2014-2015 Beef Veal Carcass Baseline Survey

Microorganisms Indicator Organism [1]	Sample Collected at	Number of Samples Tested	Number of Samples Quantifiable [2]	Percent Positive	Quantitative Levels [3] Mean (Data units)	Mean Std Error	Geometric Mean	Geo Mean 95% CI	Log 10 of the Geo Mean
Aerobic Count	**Pre-Chill**	272	204	75.00%	21,548	7,327	292.0	(198 – 429)	2.46
Enterobacteriaceae	**Pre-Chill**	273	90	32.97%	700	321	15.6	(12.6 – 19.3)	1.19
Total Coliforms	**Pre-Chill**	274	85	31.02%	19,976	19,616	14.0	(11.3 – 17.2)	1.15
Generic Escherichia coli	**Pre-Chill**	274	89	32.48%	323	123	13.9	(11.5 – 16.8)	1.14
Pathogenic Organism [4] [5]									
Salmonella	**Pre-Chill**	274	5	1.82%	0.28	0.00	0.28	(0.28 – 0.28)	-0.55
E. coli O157:H7	**Pre-Chill**	274	2	0.73%	0.28	0.00	0.28	(0.28 – 0.28)	-0.55
Non-O157 STEC [6]	**Pre-Chill**	274	27	9.85%	1.16	0.64	0.42	(0.29 - 0.63)	-0.37

(1) Units are MPN/100 cm^2 and limit of Detection (LOD) < 12.5 MPN/100 cm^2

(2) This is results above Limit of Detection (LOD); LOD < 12.5 MPN/100cm^2

(3) Calculations include estimations for results under LOD at LOD/2 = 6.25 MPN/ 100cm^2

(4) Calculations include results under LOD <0.5625 and estimated at LOD/2 = 0.28125 MPN/ 100cm^2

(5) Pathogens results are in MPN/100 cm2 with LOD < 0.5625 MPN/100cm2

(6) Non-O157 STEC are: O26, O45, O103, O111, O121, and O145. Table shows aggregate results.

Data Source – FSIS databases LIMS/PHIS, February 2016

Back→

Table 5. Comparison of Beef vs Veal Indicators at Post-Hide Removal Based on Values of Geometric Mean

Indicator	Beef	Veal	Comparison (*)
Aerobic Count (AC)	8,946	14,057	Different p-value = 0.0012
Enterobacteriaceae	30	95	Different p-value < 0.0001
Total Coliforms	19.7	61.3	Different p-value < 0.0001
Generic *E. coli*	40.8	79.6	Different p-value < 0.0001

(*) Non-parametric Wilcoxon / Kruskal-Wallis test
Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 6. Comparison of Beef vs Veal Indicators at Pre-Chill Based on Values of Geometric Mean

Indicator	Beef	Veal	Comparison (*)
Aerobic Count (AC)	106.8	292.0	Different p-value < 0.0001
Enterobacteriaceae	5.4	15.6	Different p-value < 0.0001
Total Coliforms	4.8	14.0	Different p-value < 0.0001
Generic *E. coli*	4.4	13.9	Different p-value < 0.0001

(*) Non-parametric Wilcoxon / Kruskal-Wallis test
Data Source – FSIS databases LIMS/PHIS, February 2016 Back →

Table 7. *Salmonella* Serotypes for Beef at Post-Hide Removal - 46 different serotypes in 371 positive samples

Salmonella serotype	Number of Isolates	Percent
Montevideo	80	21.56%
Anatum	59	15.90%
Cerro	40	10.78%
Kentucky	19	5.12%
Muenster	18	4.85%
Muenchen	17	4.58%
Infantis	15	4.04%
Newport	13	3.50%
Agona	11	2.96%
Meleagridis	8	2.16%
36 additional serotypes with presence from 1 to 7 and less than 2.00% each.	90	24.25%

Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 8. *Salmonella* Serotypes for Beef at Pre-Chill - 20 different serotypes in 46 positive samples

Salmonella serotype	Number of Isolates	Percent
Montevideo	8	17.39%
Muenchen	5	10.87%
Agona	4	8.70%
I 4,[5],12:i:-	4	8.70%
Give	3	6.52%
Infantis	3	6.52%
Typhimurium	3	6.52%
Derby	2	4.35%
Kentucky	2	4.35%
Uganda	2	4.35%
10 additional serotypes with 1 at 2.17% each	10	21.74%

Data Source – FSIS databases LIMS/PHIS, February 2016 Back →

Table 9. *Salmonella* Serotypes for Veal at Post-Hide Removal - 14 different serotypes in 33 positive samples

Salmonella Serotype	Number of Isolates	Percent
Cerro	7	21.21%
Montevideo	4	12.12%
Newport	4	12.12%
Typhimurium	3	9.09%
I 4,[5],12:i:-	2	6.06%
Kentucky	2	6.06%
Muenster	2	6.06%
Oranienburg	2	6.06%
Senftenberg	2	6.06%
Anatum	1	3.03%
Bredeney	1	3.03%
Infantis	1	3.03%
Manhattan	1	3.03%
Mbandaka	1	3.03%

Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 10. *Salmonella* Serotypes for Veal at Pre-Chill - 3 serotypes in 5 positive samples

Salmonella Serotype	Number of Isolates	Percent
Newport	2	40.00%
Typhimurium	2	40.00%
Anatum	1	20.00%

Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 11. Pathogen Positives by Strata for Beef at Post-Hide Removal

Strata	Samples	*Salmonella* Positive Samples	*E. coli* O157:H7 Positive Samples	Non-O157 STEC Positive Samples
1	322	92	10	9
2	446	157	5	29
3	600	122	10	46
Total	**1,368**	**371**	**25**	**84**

Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 12. Pathogen Positives by Strata for Beef at Pre-Chill

Strata	Samples	*Salmonella* Positive Samples	*E. coli* O157:H7 Positive Samples	Non-O157 STEC Positive Samples
1	322	2	0	1
2	446	3	0	0
3	600	41	9	9
Total	**1,368**	**46**	**9**	**10**

Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 13. Comparison of Beef vs Veal at Post-Hide Removal

At Post-Hide Removal					
Pathogens	Animal	Samples	Number of Positives	% Positive	Significant Difference? (*)
Salmonella	Beef	1,368	371	27.12%	Yes p-value < 0.0001
	Veal	274	33	12.04%	
E. coli O157:H7	Beef	1,368	25	1.83%	No p-value = 0.19
	Veal	274	2	0.73%	
Non-O157 STEC	Beef	1,368	84	6.14%	Yes p-value < 0.0001
	Veal	274	65	23.72%	

(*) Test of proportions (Pearson test) Back→

Data Source – FSIS databases LIMS/PHIS, February 2016

Table 14. Comparison of Beef vs Veal at Pre-Chill

At Pre-Chill					
Pathogens	Animal	Samples	Number of Positives	%Positive	Significant Difference? (*)
Salmonella	Beef	1,368	46	3.36%	No p-value = 0.18
	Veal	274	5	1.82%	
E. coli O157:H7	Beef	1,368	9	0.65%	No p-value = 0.89
	Veal	274	2	0.73%	
Non-O157 STEC	Beef	1,368	10	0.73%	Yes p-value < 0.0001
	Veal	274	27	9.85%	

(*) Test of proportions (Pearson test) Back→

Data Source – FSIS databases LIMS/PHIS, February 2016

Table 15. Reductions of Pathogens by product type from Post-Hide-Removal to Pre-Chill

Pathogens	Product Type	Percent Positive at Post-Hide Removal	Percent Positive at Pre-Chill	Significant Difference? (*)
Salmonella	Beef	27.10% (371/1,368)	3.36% (46/1,1368)	Yes p-value < 0.001
	Veal	12.04% (33/274)	1.82% (5/274)	Yes p-value < 0.001
E. coli O157:H7	Beef	1.83% (25/1,368)	0.65% (9/1,368)	Yes p-value = 0.004
	Veal	0.73% (2/274)	0.73% (2/274)	No p-value = 1
Non-O157 STEC	Beef	6.14% (84/1,368)	0.73% (10/1,368)	Yes p-value < 0.001
	Veal	23.72% (65/274)	9.85% (27/274)	Yes p-value < 0.001

(*) Test of proportions (Pearson test)
Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 16. Indicator bacteria for Beef, Comparison from Post-Hide Removal to Pre-Chill

Indicator bacteria	Parameters	Post-Hide-Removal	Pre-Chill	Load (log) Reduction (Post-Hide Removal - Pre-chill)	Is Pre-chill Significantly Lower from Post-Hide Removal? (**)
Aerobic Count (AC)	Mean (log)	340,972 (5.5327)	89,591 (4.9522)	251,381 (0.5805)	Yes p-value < 0.001
	Median (log)	9,687 (3.9861)	43.12 (1.6346)	9,643 (2.3515)	
	Geometric Mean (log)	8,945 (3.9516)	106 (2.0288)	8,839 (1.9228)	
Enterobacteriaceae	Mean (log)	17,724 (4.2485)	379 (2.5786)	17,345 (1.6699)	Yes p-value < 0.001
	Median (log)	20.6 (1.3138)	3.12 (0.4941)	17.48 (0.8197)	
	Geometric Mean (log)	30.02 (1.4774)	5.38 (0.7312)	24.64 (0.7462)	

Total Coliforms	Mean (log)	2,627 (3.4194)	25,528 (4.4070)	-22,901* (-0.9876)	Yes p-value < 0.001
	Median (log)	13.12 (1.1179)	3.12 (0.4941)	10.00 (0.6238)	
	Geometric Mean (log)	19.70 (1.2945)	4.78 (0.6797)	14.92 (0.6148)	
Generic *E. coli*	Mean (log)	6,472 (3.8110)	452 (2.6551)	6,020 (1.1559)	Yes p-value < 0.001
	Median (log)	26.87 (1.4292)	3.12 (0.4941)	23.75 (0.9351)	
	Geometric Mean (log)	40.86 (1.6113)	4.41 (0.6445)	36.45 (0.9668)	

(*) The distribution of Total Coliforms at pre-chill has a few outliers that move the mean value way to the right.
(**) Non-parametric Wilcoxon / Kruskal-Wallis test (Rank Sums). Back→
Data Source – FSIS databases LIMS/PHIS, February 2016

Table 17. Indicator bacteria for Veal, Comparison from Post-Hide Removal to Pre-Chill

Indicator bacteria	Parameters	Post-Hide-Removal	Pre-Chill	Load Reduction (post-hide-removal - Pre-chill)	Is Pre-chill Significantly Lower from Post-Hide-Removal? (*)
Aerobic Count (AC)	Mean (log)	575,654 (5.7601)	21,548 (4.3334)	554,116 (1.4267)	Yes p-value < 0.001
	Median (log)	13,750 (4.1383)	225 (2.3521)	13,525 (1.7862)	
	Geometric Mean (log)	14,057 (4.1478)	292 (2.4654)	13,765 (1.6824)	
Enterobacteriaceae	Mean (log)	5,705 (3.7562)	700 (2.8451)	5,005 (0.9111)	Yes p-value < 0.001
	Median (log)	48.12 (1.6823)	6.25 (0.7958)	41.87 (0.8865)	
	Geometric Mean (log)	95.03 (1.9778)	15.65 (1.1945)	79.38 (0.7833)	

Total Coliforms	Mean (log)	22,939 (4.3605)	19,976 (4.3005)	2,963 (0.0600)	Yes p-value < 0.001
	Median (log)	26.25 (1.4191)	6.25 (0.7958)	20.00 (0.6233)	
	Geometric Mean (log)	61.34 (1.7877)	14.00 (1.1463)	47.34 (0.6414)	
Generic *E. coli*	Mean (log)	5,592 (3.7475)	323 (0.3996)	5,269 (3.3479)	Yes p-value < 0.001
	Median (log)	40 (1.6020)	6.25 (0.7958)	33.75 (0.8062)	
	Geometric Mean (log)	79.59 (1.9009)	13.91 (1.1433)	65.91 (0.7576)	

(*) Non-parametric Wilcoxon / Kruskal-Wallis test (Rank Sums).
Data Source – FSIS databases LIMS/PHIS, February 2016

Back→

Table 18. Beef at Post-Hide-Removal Pathogen Percent Positive by Animal Class (Combined Animal Groups)

Pathogens	Animal Class	Samples	Positives	Percent Positives	Is there significant difference among classes?
Salmonella	Cow/Bull	195	55	28.21%	Yes, Dairy Cows present a higher concentration (p-value = 0.02) vs Steer/Heifer
	Dairy Cow	219	73	33.33%	
	Steer/Heifer	954	243	25.47%	
E. coli O157:H7	Cow/Bull	195	2	1.03%	No significant difference among classes (p-value = 0.17)
	Dairy Cow	219	2	0.91%	
	Steer/Heifer	954	21	2.20%	
Non-O157 STEC	Cow/Bull	195	16	8.21%	No significant difference among classes (p-value = 0.10)
	Dairy Cow	219	18	8.22%	
	Steer/Heifer	954	50	5.24%	

Data Source – FSIS databases LIMS/PHIS, February 2016

Back→

Table 19. Beef at Pre-Chill Pathogen Percent Positive by Animal Class (Combined Animal Groups)

Pathogens	Animal Class	Samples	Number of Positives	Percent Positive	Is there significant difference among classes?
Salmonella	Cow/Bull	195	8	4.10%	Yes, Dairy Cows present a higher concentration (p-value = 0.02) vs Steer/Heifer
	Dairy Cow	219	13	5.94%	
	Steer/Heifer	954	25	2.62%	
E. coli O157:H7	Cow/Bull	195	1	0.51%	No significant difference among classes (p-value = 0.63)
	Dairy Cow	219	2	0.91%	
	Steer/Heifer	954	6	0.63%	
Non-O157 STEC	Cow/Bull	195	1	0.51%	No significant difference among classes (p-value = 0.53)
	Dairy Cow	219	1	0.46%	
	Steer/Heifer	954	8	0.84%	

Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 20. Veal at Post-Hide-Removal Pathogen Percent Positive by Animal Class*

Pathogens	Animal Class	Samples	Number of Positives	Percent Positives	Is there significant difference among classes?
Salmonella	Bob-veal	97	29	29.90%	Yes, Bob-veal present a higher concentration (p-value < 0.001)
	Formula-fed veal	173	4	2.31%	
E. coli O157:H7	Bob-veal	97	0	0.00%	No significant difference between classes (p-value = 0.45)
	Formula-fed veal	173	1	0.58%	
Non-O157 STEC	Bob-veal	97	22	22.68%	No significant difference between classes (p-value = 0.85)
	Formula-fed veal	173	41	23.70%	

*Heavy calf and non-formula fed veal are not included because only four samples (2 samples per each class) were collected. Therefore the sample size is too small for a meaningful comparison.
Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 21. Veal at Pre-chill Pathogen Percent Positive by Animal Class*

Pathogens	Animal Class	Samples	Number of Positives	Percent Positives	Is there significant difference among classes?
Salmonella	Bob-veal	97	5	5.15%	Yes, Bob-veal present a higher concentration (p-value < 0.002)
	Formula-fed veal	173	0	0.00%	
E. coli O157:H7	Bob-veal	97	1	1.03%	No significant difference between classes (p-value = 0.18)
	Formula-fed veal	173	0	0.00%	
Non-O157 STEC	Bob-veal	97	9	9.28%	No significant difference between classes (p-value = 0.76)
	Formula-fed veal	173	18	10.40%	

*Heavy calf and non-formula fed veal are not included because only four samples (2 samples per each class) were collected. Therefore the sample size is too small for a meaningful comparison.
Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 22. Non-O157 STEC on Veal at Post-Hide Removal

Pathogen	Number of Isolates(*)	Overall Percent Positive
O26	11	(11/274) 4.01%
O45	2	(2/274) 0.73%
O103	36	(36/274) 13.14%
O111	23	(23/274) 8.39%
O121	0	(0/274) 0.00%
O145	1	(1/274) 0.36%

(*) There are 65 Non-O157 STEC positives at post-hide-removal; there were 8 samples with two different O-groups identified in the same sample.
Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 23. Non-O157 STEC on Veal at Pre-Chill

Pathogen	Number of Isolates (*)	Overall Percent Positive
O26	3	(3/274) 1.09%
O45	1	(1/274) 0.36%
O103	16	(16/274) 5.84%
O111	8	(8/274) 2.92%
O121	1	(1/274) 0.36%
O145	1	(1/274) 0.36%

There are 27 non-O157 STEC positives; there were 3 samples that had two different O-groups identified from the same sample.
Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 24. Pathogen Positives per Establishment for Beef at Post-Hide Removal

Plant ID	Samples per Establishment	*Salmonella* Positive (%)	*E. coli* O157:H7 Positive (%)	Non-O157 STEC Positive (%)	*Salmonella* total Contribution (%)
1	31	25 (80.65%)	1 (3.23%)	0 (0.00%)	25/371 (6.74%)
2	21	18 (85.71%)	1 (4.76%)	3 (14.29%)	18/371 (4.85%)
3	23	15 (65.22%)	0 (0.00%)	1 (4.35%)	15/371 (4.04%)
4	29	13 (44.83%)	3 (10.34%)	0 (0.00%)	13/371 (3.50%)
5	21	12 (57.14%)	0 (0.00%)	1 (4.76%)	12/371 (3.23%)
6	22	10 (45.45%)	0 (0.00%)	0 (0.00%)	10/371 (2.70%)
7	20	10 (50.00%)	0 (0.00%)	2 (10.00%)	10/371 (2.70%)
8	22	9 (40.91%)	2 (0.09%)	0 (0.00%)	9/371 (2.43%)
9	34	9 (26.47%)	2 (5.88%)	2 (5.38%)	9/371 (2.43%)
10	18	9 (50.00%)	0 (0.00%)	0 (0.00%)	9/371 (2.43%)
All others(*)	1,127	241 (21.34%)	16 (1.41%)	75 (6.65%)	241/371 (64.95%)
Total	**1,368**	**371**	**25**	**84**	**371/371 (100.00%)**

(*) There are 82 establishments with 8 to 1 positive for *Salmonella*
Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 25. Pathogen Positives per Establishment for Beef at Pre-Chill

Plant ID	Samples per Establishment	*Salmonella* Positive (%)	*E. coli* O157:H7 Positive (%)	Non-O157 STEC Positive (%)	*Salmonella* total Contribution (%)
A	5	3 (60.00%)	0 (0.00%)	0 (0.00%)	3/46 (6.50%)
B	5	3 (60.00%)	0 (0.00%)	0 (0.00%)	3/46 (6.50%)
C	6	2 (33.30%)	1 (16.70%)	1 (16.70%)	2/46 (4.35%)
D	7	2 (28.60%)	1 (14.30%)	0 (0.00%)	2/46 (4.35%)
E	7	2 (28.60%)	1 (14.30%)	0 (0.00%)	2/46 (4.35%)
F	9	2 (22.20%)	0 (0.00%)	1 (11.10%)	2/46 (4.35%)
G	20	2 (10.00%)	0 (0.00%)	0 (0.00%)	2/46 (4.35%)
H	5	2 (40.00%)	0 (0.00%)	0 (0.00%)	2/46 (4.35%)
I	7	2 (28.60%)	0 (0.00%)	0 (0.00%)	2/46 (4.35%)
J	8	2 (25.00%)	0 (0.00%)	0 (0.00%)	2/46 (4.35%)
All others(*)	1,289	24 (1.86%)	6 (0.47%)	8 (0.62%)	24/46 (52.20%)
Total	**1,368**	**46**	**9**	**10**	**46/46 (100.00%)**

(*) There are 24 establishments with 1 positive for *Salmonella*
Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 26. Pathogen Positives per Establishment for Veal at Post-hide Removal

Plant ID	Samples per Plant	*Salmonella* Positive (%)	*E. coli* O157:H7 Positive (%)	Non-O157 STEC Positive (%)	non-O157 STEC total Contribution (%)
11	33	9 (27.27%)	0 (0.00%)	6 (18.18%)	6/65 (9.23%)
12	30	8 (26.67%)	1 (3.33%)	4 (13.33%)	4/65 (6.15%)
13	31	5 (16.13%)	0 (0.00%)	11 (35.48%)	11/65 (16.90%)
14	9	5 (55.56%)	0 (0.00%)	2 (22.22%)	2/65 (3.08%)
15	31	4 (12.90%)	0 (0.00%)	11 (35.48%)	11/65 (16.90%)
16	3	1 (33.33%)	0 (0.00%)	1 (33.33%)	1/65 (1.54%)
17	15	1 (6.67%)	0 (0.00%)	3 (20.00%)	3/65 (4.62%)
18	19	0 (0.00%)	0 (0.00%)	5 (26.32%)	5/65 (7.69%)
19	16	0 (0.00%)	0 (0.00%)	3 (18.75%)	3/65 (4.62%)
20	24	0 (0.00%)	0 (0.00%)	2 (8.33%)	2/65 (3.08%)
21	33	0 (0.00%)	1 (3.03%)	8 (24.24%)	8/65 (12.31%)
22	30	0 (0.00%)	0 (0.00%)	9 (30.00%)	9/65 (13.85%)
Total	**274**	**33**	**2**	**65**	**100.00%**

Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 27. Pathogen Positives per Establishment for Veal at Pre-chill

Plant ID	Samples per Plant	*Salmonella* Positive (%)	*E. coli* O157:H7 Positive (%)	Non-O157 STEC Positive (%)	non-O157 STEC total Contribution (%)
K	16	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
L	20	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
M	30	0 (0.00%)	0 (0.00%)	2 (6.60%)	2/27 (7.40%)
N	24	0 (0.00%)	0 (0.00%)	3 (12.50%)	3/27 (11.10%)
O	9	2 (22.20%)	0 (0.00%)	1 (11.10%)	1/27 (3.70%)
P	30	0 (0.00%)	0 (0.00%)	9 (30.00%)	9/27 (33.40%)
Q	3	0 (0.00%)	0 (0.00%)	1 (33.30%)	1/27 (3.70%)
R	33	0 (0.00%)	0 (0.00%)	1 (3.00%)	1/27 (3.70%)
S	15	1 (6.60%)	0 (0.00%)	1 (6.60%)	1/27 (3.70%)
T	31	0 (0.00%)	0 (0.00%)	2 (6.45%)	2/27 (7.40%)
U	30	1 (3.300%)	2 (6.60%)	3 (10.00%)	3/27 (11.10%)
V	33	1 (3.00%)	0 (0.00%)	4 (12.10%)	4/27 (14.80%)
Total	**274**	**5**	**2**	**27**	**100.00%**

Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 28. Interventions Applied to Beef Carcasses at Post-Hide Removal

Interventions	Number of Carcasses Treated	Percent of Use
Dehairing	26	2.71%
Bacteriophages	29	3.02%
Hot water washing	104	10.83%
Medium water washing	47	4.90%
Cold water washing	255	26.56%
Caustic soda	121	12.60%
Chlorine	173	18.02%
Lactic acid hide-on carcass wash	50	5.21%
Acetic acid hide-on carcass wash	1	0.10%

Peroxyacetic acid (PAA) hide-on carcass wash	29	3.02%
Other	125	13.02%
None	725*	*
Total Interventions excluding "None"*	**960**	**100.00%**

Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 29. Pathogens in Beef Carcasses at Post-Hide Removal Divided by Treated vs Untreated

Group	Number of Samples	*Salmonella* positive (% positive) samples	*E. coli* O157:H7 positive (% positive) samples	Non-O157 STEC positive (% positive) samples
Treated	643	166 (25.80%)	15 (2.30%)	32 (5.00%)
Untreated	725	205 (28.30%)	10 (1.40%)	52 (7.20%)
Total	**1,368**	**371**	**25**	**84**

Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 30. Pathogens in Beef Carcasses at Pre-Chill Divided by Treated vs Untreated at Post-Hide Removal

Group	Sample (*)	*Salmonella*	*E. coli* O157:H7	Non-O157 STEC
Treated	501	11 (2.20%)	2 (0.40%)	4 (0.80%)
Untreated	635	28 (4.40%)	7 (1.10%)	5 (0.80%)
Total	**1,136**	**39**	**9**	**7**

(*) There are 232 samples that lost the carcass match from post-hide-removal to pre-chill and were excluded from the comparison.

Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 31. Interventions Applied to Beef Carcasses at Pre-Chill

Interventions	Number of Carcasses Treated	Percent of Use
1 Steam vacuum (hand held steam vacuum) (temperature specific)	496	12.30%
2 Lactic acid carcass wash cabinet	539	13.36%
3 Acetic acid carcass wash cabinet	21	0.52%
4 Peroxyacetic acid (PAA) carcass wash cabinet	306	7.59%
5 Hypobromous acid carcass wash cabinet	38	0.94%
6 Lactic acid carcass hand-held application	442	10.96%
7 Acetic acid carcass hand-held application	51	1.26%
8 Peroxyacetic acid (PAA) hand-held application	86	2.13%
9 Hypobromous acid hand-held application	3	0.07%
10 Other antimicrobial carcass wash (cabinet or hand-held application)	164	4.07%
11 Steam cabinets	195	4.83%
12 Hot water carcass wash (temperature specific)	628	15.57%
13 Trimming	810	20.08%
14 Chlorinated water	88	2.18%
15 None	20	0.50%
16 Other	147	3.64%
Total interventions (including "None")	**4,034**	**100.00%**

Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Table 32. Interventions Applied to Veal Carcasses at Post-Hide Removal

Interventions	Number of Carcasses Treated	Percent of Use
Dehairing	2	0.66%
Bacteriophages	0	0.00%
Hot water washing	80	26.32%
Medium water washing	41	13.49%
Cold water washing	50	16.45%
Caustic soda	0	0.00%
Chlorine	44	14.47%
Lactic acid hide-on carcass wash	6	1.97%
Acetic acid hide-on carcass wash	0	0.00%
Peroxyacetic acid (PAA) hide-on carcass wash	13	4.28%
Other	68	22.37%
None*	57*	21%*
Total Interventions excluding "None" *	**304**	**100.00%**

Data Source – FSIS databases LIMS/PHIS, February 2016

Back→

Table 33. Interventions Applied to Veal Carcasses at Pre-Chill

Interventions	Number of Carcasses Treated	Percent of Use
1 Steam vacuum (hand held steam vacuum) (temperature specific)	83	11.91%
2 Lactic acid carcass wash cabinet	50	7.17%
3 Acetic acid carcass wash cabinet	4	0.57%
4 Peroxyacetic acid (PAA) carcass wash cabinet	17	2.44%
5 Hypobromous acid carcass wash cabinet	32	4.59%
6 Lactic acid carcass hand-held application	97	13.92%
7 Acetic acid carcass hand-held application	8	1.15%
8 Peroxyacetic acid (PAA) hand-held application	42	6.03%
9 Hypobromous acid hand-held application	26	3.73%
10 Other antimicrobial carcass wash (cabinet or hand-held application)	20	2.87%
11 Steam cabinets	1	0.14%
12 Hot water carcass wash (temperature specific)	106	15.21%
13 Trimming	145	20.80%
14 Chlorinated water	15	2.15%
15 None	12	1.72%
16 Other	39	5.60%
Total interventions (including "None")	**697**	**100.00%**

Data Source – FSIS databases LIMS/PHIS, February 2016

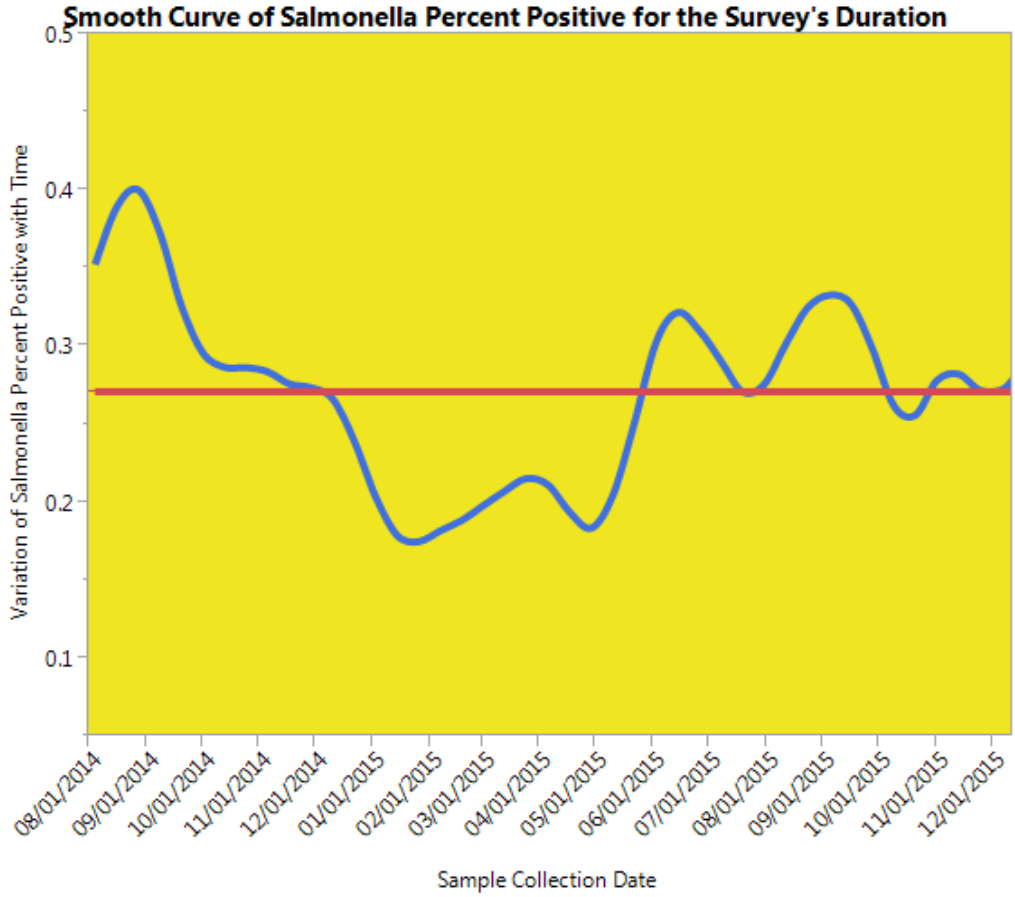
Back→

Figure 1. WesVar Output Window for *Salmonella, E. coli* O157:H7 and non-O157 STEC for Beef at Pre-Chill.

						Overall		
STATISTIC	EST_TYPE	ESTIMATE	STDERROR	LOWER 95%	UPPER 95%	CV(%)	CELL_n	DEFF
SUM_WTS	VALUE	1.0000000	0.0371051	0.9266224	1.0733777	3.7105126	1368	N/A
O157	VALUE	0.0006304	0.0004366	-0.0002331	0.0014939	69.2623099	1368	0.4139764
Salmonella	VALUE	0.0071583	0.0025158	0.0021832	0.0121334	35.1448195	1368	1.2182596
STECs	VALUE	0.0020850	0.0015302	-0.0009411	0.0051111	73.3904617	1368	1.5395195

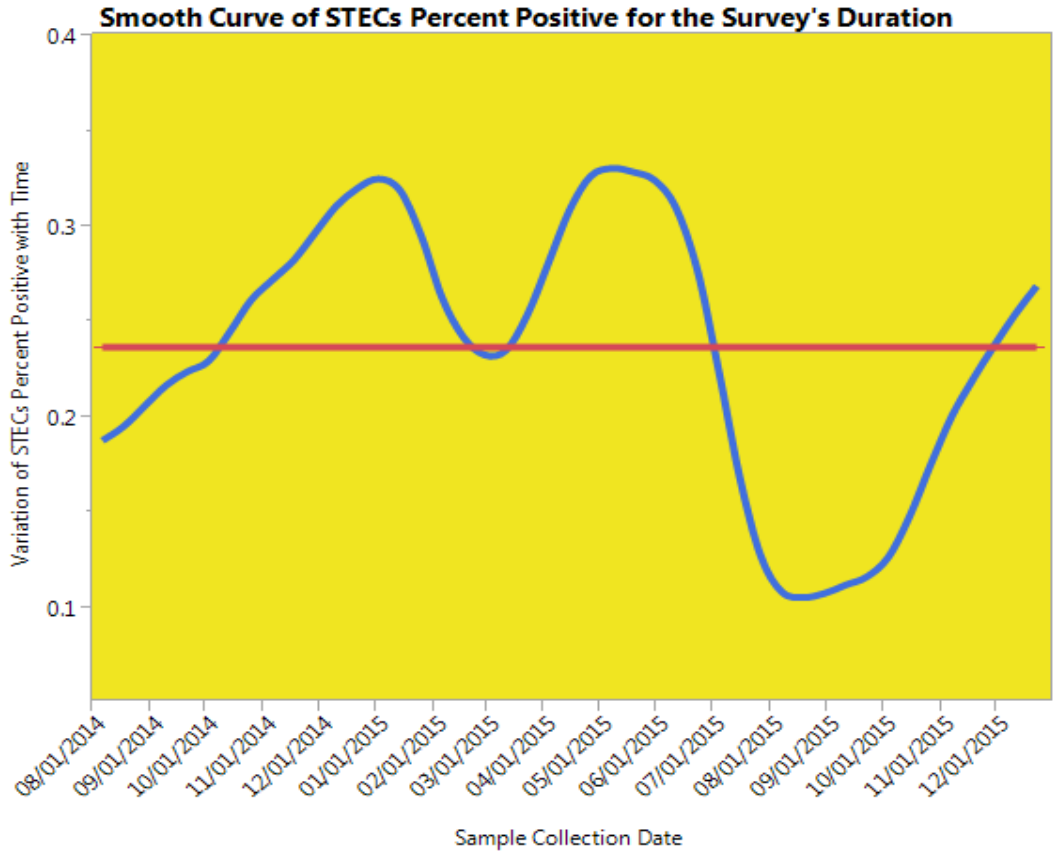
Back→

Figure 2. Change of *Salmonella* Percent Positive over Time for Beef at Post-Hide Removal



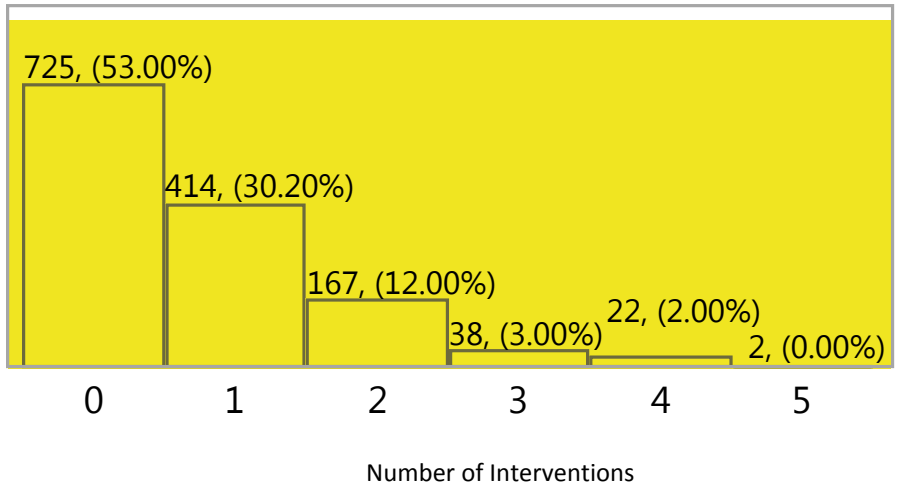
Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Figure 3. Change of non-O157 STEC Percent Positive over Time for Veal at Post-Hide Removal



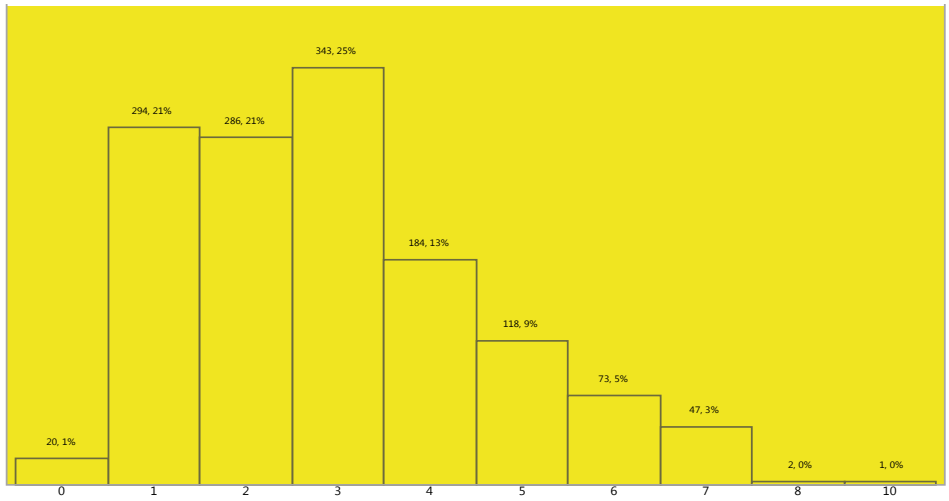
Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Figure 4. Beef Carcasses Receiving Interventions Simultaneously at Post-Hide Removal (each bar provides number of carcasses and percent it represents)



Data Source – FSIS databases LIMS/PHIS, February 2016 Back→

Figure 5. Beef Carcasses Receiving Interventions Simultaneously at Pre-Chill (each bar provides number of carcasses and percent it represents)

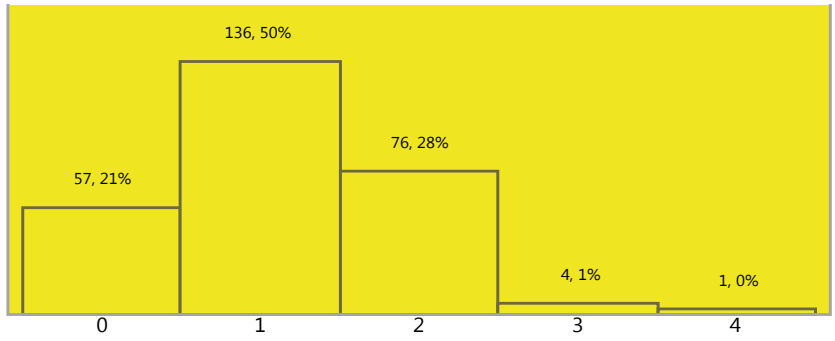


Number of interventions received

Back→

Data Source – FSIS databases LIMS/PHIS, February 2016

Figure 6. Veal Carcasses Receiving Interventions Simultaneously at Post-Hide Removal (each bar provides number of carcasses and percent it represents)

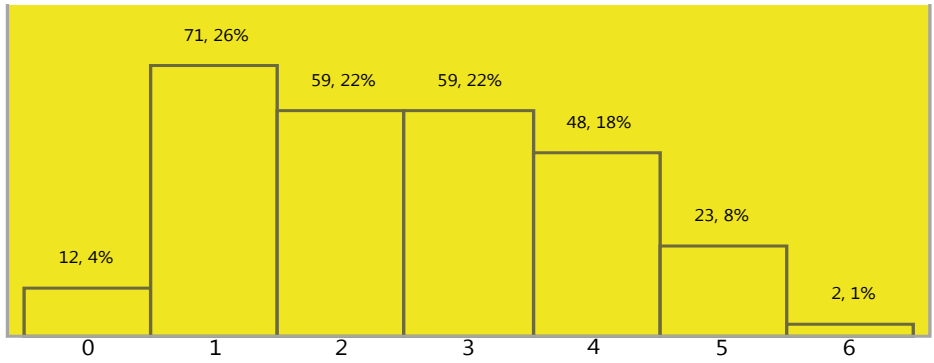


Number of Interventions

Data Source – FSIS databases LIMS/PHIS, February 2016

Back→

Figure 7. Veal Carcasses Receiving Interventions Simultaneously at Pre-Chill (each bar provides number of carcasses and percent it represents)



Data Source – FSIS databases LIMS/PHIS, February 2016

Back→

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:

Managed existing files to update total volume production of beef and veal carcasses during the survey period (17 months).

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 microorganisms had appropriate MPN values and MPN positive tube combinations and the appropriate conversion to MPN/100cm^2. The laboratories obtained serogroup and serotype information for all *Salmonella* positive samples. FSIS identified outliers for indicator organisms and corrected data entry errors; all this effort resulted on a final "official" file.

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

Merged existing files containing information about volume production to determine total production, calculated establishment and sample weight. Because the survey was extended until all sample requirements were satisfied adjustment for non-responses or missing samples was not necessary. The analyst prepared sample files for special software processing. JMP statistical software was used.

FSIS used the statistical software JMP v. 11 to merge, analyzed, build tables, compile statistical tests, produce maps, etc. and the specialized software "WesVar v 5.1" to obtain prevalence's point estimates and uncertainty.

Data

The original raw data file for BVCBS contains files with production information collected during the survey, files with answers to supplemental questions via computerized database PHIS (Public Health Information System), and lab results. Production information is essential for calculating the production during the survey and is used to calculate weight of each establishment and each sample. These files contain general information including:
FSIS collected volume information on 179 beef eligible establishments in this study. Inspectors sampled 139 of these establishments.
FSIS statistician calculated additional information about stratification and production by stratum.
The individual sample weight was calculated by integrating the production file with the survey results.
Other sections of the file showed establishment information including, plant identification number, state, stratification calculations, etc.
In addition, sample collectors' answers to supplemental questions were added to complete the files with the required information.
New variables were created to facilitate calculations.
The analyst assembled a final file with valid results for calculation of the presence and concentration of microorganisms.

Calculation of Base Sample Weights

The scope of the sampling design for the BVCBS divided the qualified beef 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 (12). As such, some sectors of the population were sampled at a higher frequency, and this type of design introduces bias. To counter-balance the bias, each sample is weighted to account for its relative impact on the result. A way to correlate the sample results and their uncertainty to all of the establishments producing beef carcasses and to estimate parameters is by using special statistical software (discussed below in the "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 (13) (14). 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 BVCBS 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* (beef carcass) 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 beef survey), each stratum is treated as an independent sample and the base 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* including establishments not sampled
$\sum V_{sj}$ is the volume of establishments that were sampled in stratum *"j"*
V_{ij} is the volume of establishment "*i*" in stratum "*j*"
Σv_j is total volume of establishments in the frame, sampled or not

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:

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

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

WesVar Statistical Procedures

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. 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 (15) (16) (17).

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 National Prevalence in Beef and Veal Carcasses for *Salmonella*, *E. coli* O157:H7 and non-O157 STEC.

Figure 10 shows the WesVar output with results for *Salmonella, E. coli* O157:H7 and **non-O157 STEC***.* Because of the use of replicated weight, this result extends to the entire production of USDA regulated beef and veal carcasses.

Back→

REFERENCES

1. FSIS Nationwide Beef and Veal Carcass Microbiological Baseline Survey - Study Design and Sampling Plan https://www.fsis.usda.gov/wps/wcm/connect/5057f4ef-f924-422c-bafe-771b1ead78e4/Beef-Veal-Carcass-Baseline-Study-Design.pdf?MOD=AJPERES

2. Food Safety and Inspection Service. Quantitative Analysis of Bacteria in Foods as Sanitary Indicators (Jan 5, 2015). MLG 3.02. Microbiology Laboratory Guidebook. Available at http://www.fsis.usda.gov/wps/wcm/connect/f2162091-af72-4888-997b-718d6592bcc9/MLG-3.pdf?MOD=AJPERES.

3. Food Safety and Inspection Service. FSIS Procedure for the Use of a Polymerase Chain Reaction (PCR) Assay for Screening *Salmonella* in Raw Meat, Carcass Sponge Samples, Whole Bird Rinses, Ready-To-Eat Meat and Poultry Products, Catfish, and Pasteurized Egg Products (Jun 29, 2014). MLG 4C.06. Microbiology Laboratory Guidebook. Available at: http://www.fsis.usda.gov/wps/wcm/connect/b7f78261-7977-4d00-9c1f-ded224560058/MLG-4C.pdf?MOD=AJPERES

4. Food Safety and Inspection Service. Isolation and Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg, and Catfish Products (Jun, 29, 2014). MLG 4.08. Microbiology Laboratory Guidebook. Available at: http://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/MLG-4.pdf?MOD=AJPERES

5. Food Safety and Inspection Service. FSIS Procedure for the Use of *Escherchia coli* O157:H7 Screening Tests for Meat Products and Carcass and Environmental Sponges (Jun 29, 2014). MLG 5A.04. Microbiology Laboratory Guidebook. Available at: http://www.fsis.usda.gov/wps/wcm/connect/6d1b7f96-1db7-4b0d-8081-e861206aedab/MLG-5A.pdf?MOD=AJPERES.

6. Food Safety and Inspection Service. Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges (Jan 15, 2015). MLG 5.09. Microbiology Laboratory Guidebook. Available at: http://www.fsis.usda.gov/wps/wcm/connect/51507fdb-dded-47f7-862d-ad80c3ee1738/MLG-5.pdf?MOD=AJPERES

7. Food Safety and Inspection Service. Detection and Isolation of non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) from Meat Products and Carcass and Environmental Sponges (Jun 29, 2014). MLG 5B.05. Microbiology Laboratory Guidebook. Available at: http://www.fsis.usda.gov/wps/wcm/connect/7ffc02b5-3d33-4a79-b50c-81f208893204/MLG-5B.pdf?MOD=AJPERES

8. Food Safety and Inspection Service. Most Probable Number Procedures and Tables 28 (Jun 29, 2014). MLG Appendix 2.05. Microbiology Laboratory Guidebook. Available at: http://www.fsis.usda.gov/wps/wcm/connect/8872ec11-d6a3-4fcf-86df-4d87e57780f5/MLG-Appendix-2.pdf?MOD=AJPERES.

9. JMP, Statistical software Version 9. SAS Institute Inc., Cary, NC, 1989-2007. Available at: http://www.jmp.com/software/.

10. WesVar. Westat Inc., Rockville, MD. Available at: http://www.westat.com/expertise/information_systems/WesVar/index.cfm.

11. "FSIS Compliance Guideline for Minimizing the Risk of Shiga Toxin-Producing Escherichia Coli (STEC) and *Salmonella* in Beef (including Veal) Slaughter Operations", found at: https://www.fsis.usda.gov/wps/wcm/connect/1c7b15f7-2815-41d4-9897-2b0502d98429/Compliance-Guideline-STEC-Salmonella-Beef-Slaughter.pdf?MOD=AJPERES

12. Cochran, W. G. 1977. Sampling Techniques. 3rd Edition. John Wiley & Sons, New York.

13. Brick, J.M. and Kalton, G. 1996. Handing Missing Data in Survey Research. Statistical methods in Medical Research, 5:215–238.

14. WesVar Manual including appendixes A, B, C, and D. 2007. Available at: http://www.westat.com/Westat/pdf/wesvar/WV_4-3_Manual.pdf.

15. Allender, H. 2007.Test of Hypothesis Using WesVar Regression Model Applied to NHANES Data. Paper/presentation at the 26th Annual National Conference of Environmental Quality Systems. Available at: http://epa.gov/quality/qs-docs/26-abstracts.pdf.

16. Allender, H. 2005. Evaluation Using WesVar of the Different Replication Methods Between NHANES 1999-2000 and NHANES 2001-2002. Paper/presentation at the 24th Annual National Conference on Environmental Quality Systems.

17. Allender, H. 2004. Determination of Asymmetric Confidence Intervals for Non-Random Samples using WesVar software. Paper/presentation at the 23rd Annual National Conference on Environmental Quality Systems.