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National Prevalence Estimate of Pathogens in Domestic Beef Manufacturing Trimmings (Trim)

December 2005 – January 2007

EDITOR'S NOTE

FSIS has updated the report, "Nationwide Microbiological Baseline Data Collection Program for Raw Ground Beef Components: Domestic Beef Trimmings." The report, which was originally posted in May 2008, was revised to include a calculation for the National Prevalence Estimate for *Escherichia coli* O157:H7 and *Salmonella.* FSIS released the new report, titled "National Prevalence Estimate of Pathogens in Domestic Beef Manufacturing Trimmings (Trim)" which includes the methods used to calculate the pathogen estimates and provides additional information on the statistical procedures used in the study.

FOREWORD

This publication is a compilation of data obtained from the Nationwide Microbiological Baseline Data Collection Program for Domestic Beef Trimmings (trim) from December 2005 – January 2007. The program was designed and performed by the Food Safety and Inspection Service (FSIS) to determine the presence and levels of indicator bacteria and estimate the prevalence of microbiological pathogens in beef trim destined to become raw ground beef. The design and implementation of this study was the result of the contribution of many offices and staff members from FSIS in the United States Department of Agriculture. The Microbiological Analysis and Data Branch, Division of Microbiology, Office of Public Health Science conducted this study and prepared this report. The microbiological analyses for this study were conducted by the Field Services Laboratories of FSIS and by the contract laboratory Food Safety Net Service, Ltd., San Antonio, TX. The collection of samples was the responsibility of the FSIS Office of Field Operations (OFO).

NATIONAL PREVALENCE ESTIMATE OF PATHOGENS IN DOMESTIC BEEF TRIMMINGS DECEMBER 2005 – JANUARY 2007

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NATIONAL PREVALENCE ESTIMATE OF PATHOGENS IN DOMESTIC BEEF MANUFACTURING TRIMMINGS (TRIM) DECEMBER 2005 – JANUARY 2007

EXECUTIVE SUMMARY

From December 2005 to January 2007, domestic beef manufacturing trimmings (trim) were collected at establishments operating under Federal Inspection. Samples were analyzed to estimate the percent positive and levels of *Salmonella*, generic *Escherichia coli*, Aerobic Plate Count (APC), *Enterobacteriaceae*, total coliforms, and *E. coli* O157:H7. The sampling frame included 250 establishments that slaughtered steers/heifers, cows/bulls, and calves under Federal Inspection and produced trim for use in raw ground beef production. Two sets of trim samples were collected during each sampling event. One set was analyzed at the FSIS Field Services Laboratories for the presence and levels of *E. coli* O157:H7. The other set was analyzed at a contract laboratory for the presence and levels of the other listed bacterial targets. This report provides an overview of this baseline study and the microbiological data results derived from beef trim sampled during this thirteen-month timeframe.

This study found that *E. coli* O157:H7 sample percent positive was 0.68% and 1.28% for *Salmonella*. In addition, the national prevalence estimate was calculated by weighting the samples to account for establishment production volume. These estimates are impacted by volume weighting and adjusted for production volume from beef trim establishments that did not participate in the baseline sample collection. Therefore, national prevalence estimates should not be compared directly with the percent positives obtained from the sample results. National prevalence estimate should not be viewed as an exact number, but as a number within a range (or interval) that has a high probability (95%) of containing the true value of prevalence. The estimated national prevalence of *E. coli* O157:H7 in beef trim is 0.39%, with a 95% confidence interval between 0.05% and 0.73%. The estimated national prevalence of *Salmonella* in beef trim is 0.78%, with a 95% confidence interval between 0.29% and 1.27%.

INTRODUCTION

The Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) is responsible for the enforcement of the Federal Meat Inspection Act, the Poultry Products Inspection Act, and the Egg Products Inspection Act. These Acts empower the Agency to appraise establishments for evidence of unhygienic conditions, to inspect raw and final products for evidence of adulteration, and to review records and production practices for the adherence

to Hazard Analysis and Critical Control Point (HACCP) regulations. In addition, the Secretary of Agriculture is authorized to conduct special assessments (baseline studies) for qualitative and quantitative levels of pathogens and indicator bacteria in raw products. In contrast to the risk-based format of FSIS HACCP verification programs, baseline studies are statistically designed to assess the industry as a whole by weighting sampling of each establishment according to its relative production volume. Data collected during baseline studies is essential for meeting the mission-critical needs of trend analysis, performance criteria, and risk assessments. Because the data are weighted by production volume, quantitative pathogen data from baseline studies provide the scientific basis for exposure assessment. Establishing microbiological criteria or standards, as well as assessing the seasonal and regional variability in prevalence and levels of pathogen and indicator bacteria are critical components of risk assessment. Baseline studies are performed independently from the verification regulatory activities of FSIS. However, the Nationwide Microbiological Baseline Data Collection Program for the Raw Ground Beef Component: Domestic Beef Trimmings did include a regulatory component due to the testing for *E. coli* O157:H7. This pathogen is considered an adulterant when detected in trim, which will result in regulatory action by FSIS.

FSIS took several steps to assure the quality of this study:

1. Implemented the “Baseline Study Questions” mailbox where the Office of Field Operations (OFO) inspection program personnel (IPP) could submit questions about the study;

2. Provided IPP with a training CD titled “Nationwide Raw Ground Beef Component Microbiological Baseline Data Collection Program Trim and Subprimal Sample Collection Training”;

3. Used FSIS Notices to provide IPP information about the study and instructions for sampling;

4. Recognized that IPP were not collecting samples as instructed in the FSIS Notice during the 90-day training phase of the project and made necessary adjustments before the actual study began in December 2005; and

5. Used a substitute establishment from the same stratum when it was reported that the requested trim product was not produced in the establishment originally in the study. Strata were based on the number of cattle slaughtered by the establishments (see Appendix A, Stratified Design). Substitutions could not be done for strata where all establishments were initially included in the study.

OBJECTIVES

This baseline study had four primary objectives:

1. To collect microbiological data from trim samples in order to determine the presence and levels of specific microbiological targets, including the human pathogens *E. coli* O157:H7 and *Salmonella*;

2. To determine a national prevalence of *E. coli* O157:H7 and *Salmonella* in beef trim. National prevalence estimates were achieved by weighting Beef Trim Baseline Study results by relative production volume;

3. To provide data for use in risk assessments, which inform risk management decisions; and

4. To provide public health information that can be used as guidance when new trim regulatory programs are being designed.

Program Design Relative to Objectives

This baseline establishes the first assessment of the presence and levels of the bacterial targets in trim used in the manufacture of raw ground beef. Analytical results for *Salmonella*, *E. coli* O157:H7 and indicator bacteria are expressed as percentage of positive samples for each bacterial target and as colony forming units (cfu) per gram of trim analyzed.

MATERIALS AND METHODS

Baseline Study

Establishments Included in the Sampling Frame

The sampling frame was derived from a 2003 survey list of establishments that reported producing trim. The initial sample frame contained approximately 250 eligible establishments that slaughtered and fabricated carcasses into trim that would be available for use in the production of raw ground beef. When the sampling frame was assembled, trim production volume information was not being collected by FSIS, so the sampling frame of eligible establishments was stratified by the number of cattle slaughtered by each establishment. There were three strata for this study: small, medium and large. This baseline study did not include samples obtained from head meat, organ meat, Advanced Meat Recovery product, or trimmings destined for such products as finely textured beef

or partially defatted chopped beef. The term "beef trimmings" included subprimal cuts such as boneless chuck or parts of boneless chuck that are frequently used as components of raw ground beef. The key to whether or not a specific subprimal was included in the beef trimmings was how it was produced and handled in the establishment. Thus, if combo bins of boneless chuck were processed and tested as trimmings by the establishment, they were to be counted as trimmings.

This baseline study included beef trim produced at federally inspected[1] establishments that slaughter cattle and bone out carcasses to produce various parts of carcasses including trimmings that are the primary component of raw ground beef. FSIS was aware that there are processing facilities that purchase parts of carcasses and produce trimmings during the normal course of cutting beef into steaks and roasts for retail and institutional markets. This type of trim is referred to as “bench trim” (1). Bench trimmings are also produced at retail stores and food service facilities, but this category of trim was not included in this study.

Deciding where to collect samples was complex. Ideally, the study would cover all raw source materials used for raw ground beef production. However, a key element in designing a microbial baseline is to ensure that, as much as possible, samples have been collected and handled in the same manner. For instance, some “downstream” trimmings are produced days or even weeks after the carcass was initially fabricated. Without knowing age and temperature history of such trimmings, there was no way to account for possible microbial growth which might bias the results. Therefore, this study focused on the large volume of trimmings produced directly after carcass chilling. All samples were shipped the day they were collected. This helped ensure that all samples had consistent time and temperature histories when they arrived at the laboratory for analysis.

Sample Design

Factors considered in the design of the sampling program include the size and variability of the target sample population, the virulence and number of microorganisms to be investigated, the practicality and limitations of sampling, and the specific data to be collected. Another factor considered in the design of the program was the projected prevalence of the pathogens in the commodity. When this baseline study began, there was no available information on the incidence of *E. coli* O157:H7 in beef trim. The agency used raw ground beef as a reference and assumed that the prevalence would be similar in trim.

[1] The study did not include imported trim or trimmings produced at State inspected facilities or custom exempt facilities that both slaughter cattle and fabricate carcasses.

Both sampling and non-sampling errors can affect the reliability of results and had to be considered in designing this program. Sampling errors occur because observations are derived from a portion rather than from the entire population; non-sampling errors can be attributed to many sources inherent in sample collection process, laboratory analysis, and processing of data. Both sampling and non-sampling errors were considered when determining the sample size.

A sufficient number of samples were essential to determine statistically significant differences in the baseline study. This study required approximately 2,000 analyzed samples to obtain reasonable levels of precision based on the projected prevalence for the bacterial targets. To achieve this number, the establishments were over-sampled to account for discarded samples.

In August 2003, over 300 questionnaires concerning volume and production details of raw ground beef components were sent to inspection program personnel at slaughter establishments. These data, in conjunction with fiscal year (FY) 2003 slaughter totals, were used to develop the sampling design of this baseline study.

For FY 2004, Congress appropriated funds for FSIS to initiate a program of recurring baseline studies that would be conducted by a contract laboratory. For this study, FSIS awarded a contract to Food Safety Net Services, Ltd., San Antonio, Texas. The beef trim baseline study would be the first baseline study conducted using a contract laboratory. The Food Safety Net Services performed all associated microbiological analyses, with the exception of *E. coli* O157:H7 tests. Since a positive *E. coli* O157:H7 test in beef trim for use in raw ground beef had regulatory consequences, these tests were performed at one of the three FSIS laboratories. Using contract and FSIS laboratories required that two samples be collected for each sampling event.

Sampling Location within the Establishment

The unit to be sampled in this baseline was one lot of trim. Due to variability in the definition of a production lot among establishments, FSIS allowed each establishment to define the size of a lot and the agency accepted the definition. Samples were taken from a lot regardless of the poundage. Furthermore, to determine the proper trim to be sampled, the establishment had to sort its beef trimmings into either (a) lots acceptable to be used in the manufacture of raw ground beef or (b) lots that could only be used in the manufacture of product with a lethality step. This program only sampled from production lots that were available or approved to be used to produce raw ground beef.

Sample Collection and Description

All samples were collected Monday through Friday during trim processing.

Samples were placed in insulated shipping containers with gel packs capable of maintaining the proper temperature, and shipped to the laboratories by an overnight delivery service on the same calendar day they were collected.

During the design phase of the study, FSIS was aware that a wide variety of sample collection methods were then being used for beef trimmings. These methods included collecting samples of the purge, collecting a sample using a core-drilling device, and several variations on collecting amounts of surface tissue from containers of trim. FSIS had many discussions with scientists from the USDA Agricultural Research Service (ARS) and scientists from industry. It was decided to use a sample collection method referred to as N60. The N60 method involves collecting 60 separate thin slices of surface tissue from a production lot. The weight of the 60 slices would equal two pounds. Of the methods reviewed, the N60 method was considered to be both (1) feasible at the point of trim production, and (2) have the highest probability of detecting *E. coli* O157:H7 if contamination were present. The N60 sampling concept was originally based on the International Commission on Microbiological Specifications for Foods (ICMSF) [(2)] Case 15 sampling plan, which was the most robust of all sampling plans recommended by ICMSF.

Two separate N60 samples were obtained from each lot. One 2-pound sample was shipped via Federal Express to the contract laboratory, Food Safety Net Services, Ltd. The project code for this sample was MM45. The second sample was shipped via Federal Express to one of the three FSIS Field Service Laboratories for analysis of the presence and levels of *E. coli* O157:H7. The project code for this sample was MM45R.

Selection of Organisms

The recommendations contained within the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) FINAL REPORT: NACMCF Response to USDA/FSIS Request For Guidance On Baseline Study Design and Evaluations For Raw Ground Beef Components[(3)] were used to guide the selection of microorganisms for analysis. Analyses of specific indicator organisms were included in this study to examine the possibility of using these bacteria as a measure of process control. The microbiological targets analyzed in the study include Aerobic Plate Count (APC), *Salmonella*, generic *E. coli*, *Enterobacteriaceae*, total coliforms, and *E. coli* O157:H7. The agency will further explore the baseline data on indicator organisms and interventions to determine its utility in developing strategies for process control and risk mitigation.

Analytical Methods

The methods of analysis used in this baseline study were derived from the FSIS Microbiology Laboratory Guidebook (MLG) and the Official Methods of Analysis of AOAC International. To analyze the samples for the indicator bacteria, a 25 gm sample of beef trim consisting of random pieces from the larger 2-pound sample was added to 225 ml of buffered peptone water (BPW) and stomached for two minutes. Dilutions from 10^{-1} to 10^{-4} were made and plated onto Petri film to enumerate *Enterobacteriaceae*, generic *E. coli*, total coliforms, and to perform the APC[4][5][6].

To analyze the samples for *Salmonella*, another 25 gm sample was added to 225 ml of BPW and stomached for two minutes. An aliquot of the homogenate was screened for *Salmonella* using the DuPont BAX system [7][8]. Samples that screened positive were then analyzed using a 9-tube Most Probable Number (MPN) procedure to estimate the levels of *Salmonella* in the sample[7][8]. *Salmonella* isolates were shipped to the USDA National Veterinary Services Laboratory for serotype determination.

To analyze the trim samples for *E. coli* O157:H7, five individual 65 gm portions were prepared using the methods as outlined in the MLG [9]. Samples that screened positive with the BAX system were reported as potential positive samples and were further analyzed per MLG 5. For enumeration by MPN, the procedure is described below:

Enumeration of *E. coli* O157:H7 in Trim by MPN

1. Aseptically weigh out a 325 gm test portion from the potential positive sample into a sterile stomacher bag.

2. Add 650 ml of mEC+n Broth, pre-warmed per MLG 5 for this and all subsequent dilutions, and stomach for two minutes.

3. Transfer 30 ml into each of 3 sterile bottles or bags to represent a 10 gm sample dilution.

4. Transfer 3 ml into each of 3 sterile tubes containing 7 ml of mEC+n Broth to represent a 1 gm sample dilution.

5. Transfer 0.3 ml into each of 3 sterile tubes containing 9.7 ml of mEC+n Broth to represent a 0.1 gm sample dilution.

6. The above steps provide for a 3-tube 3-dilution MPN (i.e., 9 individual dilutions for each potentially positive sample).

7. Analyze each dilution per MLG 5A and MLG 5.

8. MPN dilutions that screen negative will be recorded as negative.

9. MPN dilutions that screen positive will be confirmed using MLG 5.

10. Interpret the pattern of confirmed positive dilutions according to the MPN table for 10-1-0.1 gm analysis [10].

National Prevalence Estimate

The initial scope of the sampling design for the study divided the wide spectrum of United States beef-trim-producing establishments into three classes: high-volume establishments, mid-volume establishments, and low-volume establishments. These designations were based on the number of cattle slaughtered by the establishments (see Appendix A, Stratified Design). Sample collection was distributed among the three groups as 50%, 30%, and 20% for stratum 1, 2, and 3, respectively.

Collecting samples from pre-determined groups that are not of equal size, results in a sample collection scheme that is not completely random. This means that each establishment does not have an equal probability of selection among the volume strata. A purely random sampling design occurs when every establishment in the study has an equal probability of selection. In this study some segments of the population sampled were intentionally given preference over the others (i.e., more samples were collected from large establishments). In order to counter balance the design effect and accommodate for any bias, each sample was weighted to account for its relative impact on the result. To extend the sample results and its uncertainty to the entire spectrum of beef trim producing establishments, special statistical methodology was applied. This approach is discussed in Appendix B, Statistical Procedures.

National prevalence estimate procedures:

1) Examination of records to determine the volume of beef trim dedicated to the manufacture of raw ground beef. This process included the collection of beef trim production data from the FSIS sample request form, the determination of the number of samples per establishment in each month, and the calculation of sample weights. In addition, adjustments were done using data from a survey to account for establishments that were not sampled during the baseline study. These adjustments were made using a cell-crossed combination of establishment volume from 15 districts and 3 strata.

2) Estimation of uncertainty for non-random weighted samples. A two-sided confidence interval was computed using the replication method Jackknife n (JKn) for stratified designs with two or more primary sampling units (PSUs) per stratum.

Data were contained in three files:

(i) The *E. coli* O157:H7 file This file contains the laboratory results for 1,900 samples tested for *E. coli* O157:H7 and beef trim production information (extracted from the FSIS sample request form) for 159 establishments.
(ii) The *Salmonella* file This file contains the laboratory results for 1,719 samples tested for *Salmonella* for 157 sampled establishments.
(iii) The Survey file This file contains data from a survey on production volume for 612 beef-trim-producing establishments. Data were obtained from the FSIS Establishment Survey System Reporting, which is an establishment profile extension. The survey file complements the baseline study because not all beef-trim-producing establishments were sampled during the baseline study. Cross-checking revealed that 77 of these establishments were included in the sampling. As more current data became available from the survey file, FSIS set-aside the initial production data and the number of establishments that were not sampled reduced to 535. Establishments represented in the Survey file that were not sampled had small production volumes accounting for 4.4% of the total beef-trim-production volume in the United States.

During the original design of the Beef Trim Baseline Study, beef-trim-production volume in the United States was unknown. Hence, the initial stratification was based on the surrogate variable “Heads Slaughtered” by each establishment. At the beginning of the study, it was assumed that a correlation existed between the number of cattle slaughtered and the amount of trim produced by an establishment. Data on beef trim production was gathered during the course of the study. This allowed for a recalculation of the boundaries of each stratum based on the establishments monthly beef trim production. A new stratum boundary divided establishments by monthly beef trim production; there was no commingling of establishments belonging to different strata. The new boundaries were selected to maintain the same number of establishments within each original stratum without making a radical change to the original stratification design. The new beef-trim-based boundaries had a similar establishment proportion by stratum as the stratification based on number of cattle slaughtered.

Stratum	**New Boundary**
1 − Large	Over 2,200,000 pounds of beef trim per month
2 − Small	Over 73,000 but less than 2,200,000 pounds of beef trim per month
3 − Very small	Less than 73,000 pounds of beef trim per month

Each sample was weighted using information from the *E. coli* O157:H7 file by following the two-step procedure and formulas described in Appendix C, Sample Weight Calculation. This weighting used volume data extracted from block 28 of the sample request form; these sample weights were extended to the *Salmonella* file. These adjustments also accounted for establishments that were not sampled; calculations for each sample were performed to derive the adjusted weight per sample.

The sample data were prepared for the final calculations by:

1. Matching the calculated establishment weights to each establishment's identification number in the *E. coli* O157:H7 and *Salmonella* sample files;
2. Identifying the variable "strata" (1, 2, or 3) in the sample files; and
3. Assigning the PSU (primary sampling unit) within each stratum to each establishment in the sample file.

RESULTS

Analysis of 1,900 samples for the presence of *E. coli* O157:H7 revealed 13 positive samples, for a percent positive rate of 0.68 (Table 1). This value is based on a sample raw number and should not be considered as the national prevalence. Of the 13 positive samples, 12 were enumerated (Table 7). Six of the 12 samples were below the limit of detection (LOD). The range per gram of the remaining six samples above the LOD was 0.036 to 1.5 colony forming units (cfu) per gram of trim. The average number of *E. coli* O157:H7 cfu per gram was 0.56 for the six samples with MPN values.

Indicator organisms were analyzed from 1,719 samples (Table 1). The percent positive rate for the Aerobic Plate Count (APC) was 99.30 and 59.05 for *Enterobacteriaceae*. Total coliforms and generic *E. coli* had percent positive rates of 41.94 and 15.71, respectively. Quantitative distribution of the indicator organisms found in the positive beef trim samples can be located in Tables 2 – 6.

There were 1,719 samples analyzed for the presence of *Salmonella* sp. Of these, 22 tested positive, for a percent positive rate of 1.28 (Table 1). There was an average of 12.6 cfu of *Salmonella* per gram of trim for the 9 samples with MPN values. Thirteen of the 22 positive samples were below the LOD for the MPN assay. For those samples above the LOD, the range was 0.4 to 46 cfu per gram of trim (Table 8).

The *Salmonella* serotypes isolated most often from trim samples were Cerro (3) and Montevideo (3). *S.* Heidelberg, *S.* Infantis and *S.* Kentucky were each found twice. The remaining *Salmonella* serotypes were each found once: Agona,

Bredeney, Dublin, Fresno, Lille, Meleagridis, Oranienburg, Schwarzengrund, and Typhimurium (4,12:i:-).

Calculation of National Prevalence of *E. coli* O157:H7 in Beef Trim

Because of the weighting, the calculated prevalence result extends to the entire spectrum of establishments producing beef trim in the United States during the time-period of the beef trim baseline study. The weighted estimated prevalence of *E. coli* O157:H7 is 0.00387, or 0.39%. Statistical analysis based on replications provided a 95% confidence interval, which is an interval that has a 95% probability of containing the true value of the prevalence of *E. coli* O157:H7.

In summary:

- The estimated national prevalence of *E. coli* O157:H7 in beef trim is 0.39%, with a 95% confidence interval between 0.05% and 0.73%.

Calculation of National Prevalence of *Salmonella* in Beef Trim

Because of the use of weighting, the calculated prevalence result extends to the entire spectrum of establishments producing beef trim in the United States during the time-period of the beef trim baseline sampling. The weighted estimated prevalence of *Salmonella* is 0.0078, or 0.78%. Statistical analysis based on replications provided a 95% confidence interval, which is an interval that has a 95% probability of containing the true value of the prevalence of *Salmonella*.

In summary:

- The estimated prevalence of *Salmonella* in beef trim is 0.78% with a 95% confidence interval between 0.29% and 1.27%.

DISCUSSION

The Nationwide Microbiological Baseline Data Collection Program for the Raw Ground Beef Component: Domestic Beef Trimmings was designed to determine the presence and the levels of selected bacteria in beef trim produced in federally-inspected establishments and destined to be included in the production of raw ground beef. Although FSIS has conducted baselines on other beef products and beef carcasses (Cows and Bulls, Raw Ground Beef, Steers and Heifers; www.fsis.usda.gov/Science/Baseline_Data/index.asp), this baseline is the first study that examined one of at least five components that make up raw

ground beef. Thus, the data reported in this study provide new insight into the microbiological profile of this component. In addition, this baseline was the first time that the Agency used the N60 method for sample collection.

Prior to conducting this baseline study, the Agency did not have a regulatory program for testing beef trim and, as a result, trim volume information was not available. During this baseline, study volume information was collected using a survey instrument designed specifically for this purpose. In this study, 1,900 samples were analyzed for *E. coli* O157:H7 and 1,719 for *Salmonella*. These samples represent only a proportion of establishments that produce beef trim. In calculating the National Prevalence Estimate, the spectrum of trim production had to be constructed from the available survey data. This baseline study used specialized statistical procedures to perform this calculation (see Appendix C, Sample Weight Calculation).

The indicator organism data was collected and analyzed to determine the percent positive and quantitative distribution of bacteria. The agency will further explore the baseline data on indicator organisms to determine its utility in developing strategies for process control. The presence of the pathogen *E. coli* O157:H7 in trim indicated that this component is a potential source of *E. coli* O157:H7 in raw ground beef. This study was not designed to provide microbiological information on individual establishments, which would require a larger number of samples to be collected from every establishment over an extended period. Data analyses indicated that while smaller establishments have more percent positives for *Salmonella* (statistical significance P value equals 0.02), the difference among the three establishment strata did not exist for *E. coli* O157:H7 (no statistical significance P value equals 0.66).

Data obtained from baseline studies and the national prevalence calculations are useful for the Agency to establish performance standards and guidelines for the industry, as well as to perform risk assessments. The trim data collected from this baseline program provided the Agency with information that was essential for establishing regulatory policy.

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TABLES

Table 1. Percent Positive of Selected Microorganisms on Raw Ground Beef Components (Trim)

Microorganisms	Sample Positive: Samples Analyzed	Number Positive	Percent Positive	SE[3]
Indicator Organisms[1]				
Aerobic Plate Count @ 35°C	1,719	1,707	99.30	0.20
Enterobacteriaceae	1,719	1,015	59.05	1.19
Total Coliforms	1,719	721	41.94	1.19
Generic *Escherichia coli*	1,719	270	15.71	0.88
Pathogenic Organisms[2]				
Escherichia coli O157:H7[4]	1,900	13	0.68	0.19
Salmonella	1,719	22	1.28	0.27

[1] Equal to or above the detection limit
[2] Qualitative results
[3] Standard Error using binomial distribution
[4] *E. coli* O157:H7 has a higher acceptance temperature for analysis than *Salmonella*

Table 2. Mean Level of Selected Microorganisms per Gram on Raw Ground Beef Components (Trim)

Microorganisms	Number of Samples Quantified	Number of Samples Positive[1]	Level of Positives			
			Log_{10} Mean		Geometric Mean[6,7]	
			Mean	SE	Mean	95% CL
Indicator Organisms[2]						
Aerobic Plate Count @ 35°C	1,719	1,707	4.71	3.79	1,209.45	(1,074.2, 1,361.6)
Enterobacteriaceae	1,719	1,015	3.39	2.98	11.11	(9.88, 12.49)
Total Coliforms	1,719	721	3.2	2.97	5.12	(4.60, 5.69)
Generic *Escherichia coli*	1,719	270	1.9	1.7	1.69	(1.59, 1.80)
Pathogenic Organisms[3]						
Escherichia coli O157:H7	12[4]	6	-0.54	-0.86	0.07	(-)[5]
Salmonella	22	9	0.72	0.39	0.66	(0.9, 30.2)

[1] Positive by quantitative method
[2] Equal to or above the limit of detection.
[3] Mean and range by MPN method
[4] One quantitative positive sample not enumerated
[5] Insufficient numbers of positive results to calculate valid CL

[6] $Geometric\ \ mean = \mu_g = \exp(\frac{1}{n}\sum_{i=1}^{n}\ln x_i)$,

[7] $Geometric\ \ standard\ \ deviation = \sigma_g = \exp\{\sqrt{\sum_{i=1}^{n}\frac{(\ln x_i - \ln \mu_g)^2}{n}}\}$, and $SE_{geometric} \approx \frac{standard deviation of\ Log(x_i) \times Geometric Mean}{\sqrt{n-1}}$

Table 3. Distribution of APC at 35ºC

Range, cfu/g	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
<10[(1)]	12	0.7	12	0.7
10-100	206	12.0	218	12.7
101-1,000	740	43.0	958	55.7
1,001-10,000	443	25.8	1401	81.5
10,001-100,000	210	12.2	1611	93.7
100,001-1,000,000	77	4.5	1688	98.2
1,000,001-10,000,000	31	1.8	1719	100.0
TOTAL	1719	100	-	-

[(1)]Below the level of detection

Table 4. Distribution of *Enterobacteriaceae*

Range, cfu/g	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
<10[(1)]	704	40.95	704	41.0
10-100	731	42.52	1435	83.5
101-1,000	206	11.98	1641	95.5
1,001-10,000	51	2.97	1692	98.4
10,001-100,000	20	1.16	1712	99.6
100,001-1,000,000	6	0.35	1718	99.9
1,000,001-10,000,000	1	0.06	1719	100.0
TOTAL	1719	100	-	-

[(1)]Below the level of detection

Table 5. Distribution of Coliforms

Range, cfu/g	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
<10[(1)]	998	58.06	998	58.1
10-100	543	31.59	1541	89.6
101-1,000	130	7.56	1671	97.2
1,001-10,000	33	1.92	1704	99.1
10,001-100,000	12	0.70	1716	99.8
100,001-1,000,000	2	0.12	1718	99.9
1,000,001-10,000,000	1	0.06	1719	100.0
TOTAL	1719	100	-	-

[(1)]Below the level of detection

Table 6. Distribution of Generic *Escherichia coli*

Range, cfu/g	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
<10[(1)]	1449	84.29	1449	84.3
10-100	239	13.90	1688	98.2
101-1,000	20	1.16	1708	99.4
1,001-10,000	10	0.58	1718	99.9
10,001-100,000	1	0.06	1719	100.0
TOTAL	1719	100	-	-

[(1)]Below the level of detection

Table 7. Distribution of *Escherichia coli* O157:H7

Range, MPN/g	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
<0.03[(1)]	6	50.0	6	50.0
0.031-0.30	3	25.0	9	75.0
0.31-3.0	3	25.0	12	100.0
TOTAL[(2)]	12	100.0	-	-

[(1)]Below the level of detection
[(2)]One positive sample not enumerated

Table 8. Distribution of *Salmonella*

Range, MPN/g	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
<0.3[(1)]	13	59.1	13	59.1
0.31-3.0	2	9.1	15	68.2
3.01-30.0	6	27.3	21	95.5
31.0-300.0	1	4.5	22	100.0
Total	22	100.0	-	-

[(1)]Below the limit of detection

APPENDICES

Appendix A: Stratified Design

Domestic Trim Baseline Sample Design Modification

The following describes the stratification of the samples based on production volume information obtained during a survey submitted to selected slaughter establishments.

Establishments in the sampling frame were grouped into three strata, primarily based on establishment size, using Fiscal Year (FY) 2003 Electronic Animal Disposition Reporting System (eADRS) slaughter data.

Stratum 1 beef establishments that slaughter at least 100,000 head in FY03. Each plant in this stratum was scheduled to receive two sample requests each month for the duration of the study.

Stratum 2 beef establishments that slaughter between 1,000 and 100,000 head in FY03. Each selected plant in this stratum was scheduled to receive one sample request each month for the duration of the study.

Stratum 3 beef establishments that slaughter between 100 and 1,000 head in FY03 and that were identified in the raw ground beef component survey as producing domestic trim product. Each selected establishment in this stratum was scheduled to receive in total six sample requests during the period of the study.

Appendix B: Statistical Procedures

When data are collected as part of a complex sample survey, it is often difficult 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 often too small. A technique called “replication methods” provides a way to estimate variance for the types of complex sample designs and weighting procedures used 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. 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 can be extended to the entire national beef trim production.

For the particular design of our study, the methodology selected was the Jackknife n (JK_n), where the strata are groups of establishments that are sampled as if they were a separate population and the primary sampling units (PSUs) are the individual establishments within each stratum. The JK_n replication was used because it is applicable for general stratified samples in which two or more PSUs (establishments) per stratum have been defined, as was done in this study design.

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. Furthermore, the same procedure is applicable to most statistical analyses desired, such as means, percentages, ratios, or correlations. 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.

Replication calculations comprise four steps:

Step 1. Divide the sample into subsample replicates that mirror the design of the sample by specifying the variables “strata” and “PSU”.
Step 2. Calculate weights for each replicate, using the same procedures used for the full-sample weight.
Step 3. Calculate estimates for each of the replicates using the same methods used for the full-sample estimate.
Step 4. Estimate the variance of the full-sample estimate using the resulting full-sample and replicate estimates.

Appendix C: Sample Weight Calculation

The sample weight begins with a calculation of the initial sample weight for each individual sample collected in the trim baseline study. The initial sample weight (W_{ij}) for the j^{th} sample (S_{ij}) of establishment *i* was determined as follows:

$$W_{ij} = V_{ij} \times T_{ij} \times K \qquad (1)$$

where
V_{ij} is the production volume (in pounds) of beef trim available for raw ground beef produced in the LAST FULL PRODUCTION DAY (All Shifts) when sample S_{ij} was taken as reported in block 28 of the Sample Request Form,

T_{ij} is the number of days in the LAST 30 DAYS in which beef trim was produced (collected from block 28 for sample S_{ij}), and

K is a factor that accounts for the proportion of samples in a given month or the lack of it. For example,
K = 1/2, if two samples were collected in a month for a given establishment;
K = 1, if one sample was collected in a month for a given establishment;
K = 2, if one sample was collected in a two-month period for a given establishment;
K = 3, if one sample was collected in a three-month period for a given establishment, etc.

The initial sample weight $W_{ij,}$ calculated above was adjusted to account for the volumes of beef trim produced by non-participating establishments in the same district and stratum as establishment *i*. The non-participating establishments and their 30-day beef trim volumes were obtained from the FSIS production volume survey. The adjusted sample weight ($AdjW_{ij}$) for the j^{th} sample S_{ij}, of establishment *i* was determined as follows:

$$AdjW_{ij} = W_{ij} \times (1 + \Sigma N_v / \Sigma V_l) \qquad (2)$$

where
$\Sigma N_v / \Sigma V_l$ is the adjustment factor,

ΣN_v is the total beef trim volume in the 30-day period from all non-participating establishments in the same district-stratum combination as establishment *i*, and

ΣV_l is the total beef trim volume in the 30-day period calculated from the last samples for all participating establishments in the same district-stratum combination as establishment *i*. (The last sample was selected to represent the establishment because of its proximity to the month in which the survey of establishments not sampled was taken).

For example, if there were two participating establishments in a given district-stratum combination, and ΣN_v =25,000, V_1 = 4,000, and V_2 = 6,000, then

$$AdjW_{ij} = W_{ij} \times [1 + \Sigma N_v / (V_1 + V_2)] = W_{ij} \times (1+ 2.5)$$