Nationwide Sponge Microbiological Baseline Data Collection Program: Cattle

June 1997 – May 1998

FOREWORD

This publication is a compilation of data obtained from the Nationwide Sponge Microbiological Baseline Data Collection Program for Cattle for the twelve months from June 1997 – May 1998. The program was initiated by the Food Safety and Inspection Service (FSIS) to estimate the prevalence and levels of bacteria of public health concern on cattle carcasses as currently produced. The program was designed through consultation with various staffs in the Agency. The Biosciences Division (formerly the Microbiology Division) in conjunction with the Data Analysis and Statistical Support Staff (formerly the Evaluation and Analysis Division) coordinated the conduct of the program, provided data analysis and prepared this report. The microbiological analyses were conducted by the FSIS Field Service Laboratory at Athens, GA. Sample collection was the responsibility of the FSIS Inspectors-in-Charge without whose cooperation this program could not have been accomplished.

NATIONWIDE SPONGE MICROBIOLOGICAL BASELINE DATA COLLECTION PROGRAM: CATTLE JUNE 1997 – MAY 1998

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NATIONWIDE SPONGE MICROBIOLOGICAL BASELINE DATA COLLECTION PROGRAM: CATTLE JUNE 1997 – MAY 1998

EXECUTIVE SUMMARY

From June 1997 to May 1998, sponge samples from cattle carcass halves were collected at establishments operating under Federal inspection. These samples were analyzed to estimate the prevalence of *Salmonella* and the prevalence and levels of generic *Escherichia coli* on cattle carcasses as currently produced. All establishments that slaughtered steers/heifers, cows/bulls, and calves under Federal inspection during the sample collection period were included in the sampling frame. Sponge samples from 1,881 carcass halves were analyzed qualitatively for *Salmonella*, a pathogen often associated with human illness as determined by foodborne illness reports. Samples from 1,881 different carcass halves were analyzed quantitatively for generic *E. coli*, an indicator of general hygiene or process control. The estimates of national prevalence for *Salmonella* and generic *E. coli* in cattle were 1.2% and 16.6%, respectively. Generic *E. coli* of 10 or fewer colony forming units (cfu) per cm^2 were found on 98.9% of the carcass sponge samples. Generic *E. coli* are generally considered nonpathogenic.

INTRODUCTION

The Food Safety and Inspection Service (FSIS) is the Federal agency responsible for enforcing the Federal Meat Inspection Act, the Poultry Products Inspection Act and the Egg Products Inspection Act. These Acts empower the Agency to review facilities for evidence of insanitation, to inspect final products for evidence of adulteration and to review labels to assure proper product labeling. The Acts stipulate the penalties which the Agency can impose to assure compliance. The inspection of food animals at the time of slaughter has historically focused on identifying symptoms of disease conditions that make the carcass of the animal or parts of the carcass unfit for human food. Many human pathogens, however, reside harmlessly on the hide, feathers or skin of healthy animals or in their digestive tracts, just as they often reside on the skin and hair of humans, causing no symptoms of disease. Bacteria are not detectable by visual inspection. Bacteria of many types are, in fact, natural and unavoidable residents of all warm blooded animals including humans. The slaughter procedures that have developed over the years, as well as recently implemented antimicrobial interventions (e.g. trisodium phosphate, organic acid rinses, steam vacuuming, steam pasteurization, antimicrobial sprays) for various species, reduce the levels of many pathogenic microorganisms, but do not completely eliminate them. Because the production of raw meat and poultry does not include a procedure, such as cooking, that can be designed to kill remaining bacteria, any microorganism naturally found on these animals, including human pathogens, could be present on the final raw product. This fact has long been recognized by the Agency and by scientific experts worldwide.

Raw products, because they are not cooked or similarly processed, cannot be expected to be as free of pathogenic bacteria as is expected in cooked products. Even when produced under ideal conditions, carcasses, primal, sub-primal, and retail cuts of meat from normal, healthy animals contain a variety of bacteria including low levels of some pathogens. Refrigerated raw meats will eventually undergo microbial spoilage even if they are produced from the carcasses of normal, healthy animals, fabricated under good manufacturing conditions, and properly refrigerated. If red meats are not properly cooked, held, cooled, and stored, the pathogens present on these products can cause foodborne illness if the product is consumed.

OBJECTIVES

This non-regulatory program had three primary objectives:

1. To collect data by sponge sampling that provide a microbiological profile of cattle carcasses for generic *E. coli* and *Salmonella*.

2. To develop, using the sponge sampling method, generic *E. coli* and *Salmonella* guidelines in support of the Pathogen Reduction/Hazard Analysis Critical Control Point (PR/HACCP) Regulation of July 25, 1996[1]. (The use of sponge sampling for the collection of microbiological data from carcasses is an acceptable non-destructive sampling alternative that alleviates many of the problems associated with excision sampling procedures previously used to establish slaughter species baselines.)

3. To use the information and knowledge gained from this program as a reference for further investigations and evaluation of new prevention programs.

Program Design Relative to Objectives:

The Nationwide Sponge Microbiological Baseline Data Collection Program: Cattle focused on establishing a new microbiological baseline for cattle production. These results on the presence and quantity of selected microorganisms are expressed as a national average relative to slaughter volume. The data obtained provide an updated *Salmonella* and generic *E. coli* profile of cattle as currently produced under Federal inspection. This approach is similar to the previous FSIS Nationwide Microbiological Baseline Data Collection Programs for steers and heifers[2], cows and bulls[3], broilers[4], market hogs[5], and young turkeys[6].

PROGRAM DESIGN

Establishments Included in the Sampling Frame:

All establishments that slaughtered steers/heifers, cows/bulls, and calves under Federal inspection during the sample selection period were included in the sampling frame. There were approximately 735 establishments slaughtering about 29 million steers/heifers, around 650 establishments slaughtering approximately 8 million cows/bulls, and about 550 establishments slaughtering 1.7 million calves. Many of these establishments slaughter more than one class of bovines.

Sample Design:

In order to approximate a random sample, the selection was performed in two stages. The first stage was to randomly select an establishment from the sample frame, and the second was to randomly select the cattle carcass sample. Establishments were selected with probabilities in proportion to their total number

of cattle slaughtered by slaughter class. When the establishments were randomly selected to collect a sample, the slaughter class (e.g., steers/heifers, cows/bulls, or calves) was also designated. FSIS personnel subsequently collected a sample from the slaughter class designated. Establishments that have a large percentage of animals slaughtered had a greater chance of being selected, and could also be selected more than once. This procedure gave all cattle slaughtered an approximately equal chance of being selected.

It was determined that a sample size of about 2,000 samples per microorganism would ensure reasonable levels of precision for yearly estimates given the expected prevalences for the bacteria included in this study. To achieve this number (2,000) of samples, about 3,000 samples were requested during the 52 week time frame of the study (approximately 57 per week). Of these, laboratory results for each microorganism were obtained for 1,881 cattle carcass halves. Some samples were not collected for various reasons, such as the establishment did not slaughter the cattle class selected that particular week. Other samples were collected but not analyzed if they became compromised during shipment (e.g., open package, invalid temperature, delayed shipment, etc.).

Data Limitations:

The program was designed to provide estimates of national prevalences and levels for generic *E. coli* and *Salmonella* on cattle carcasses. The data obtained provide information about these organisms, which might be present on federally inspected cattle carcasses.

The program was not designed to provide microbiological information on individual establishments. In order to obtain such information, one would need to collect a large number of samples from each establishment over a period of time.

The *Salmonella* results obtained from this one-year program are independent from the Agency's ongoing *Salmonella* HACCP Testing.

Sampling Location Within the Establishment:

To accomplish the objectives of this program, data must be derived from a significant point in the production process. There are good arguments for any number of plant sampling sites[7][8][9]. Key factors in the microbial profile of cattle are the slaughter and carcass dressing processes conducted under Federal inspection. To evaluate these processes, samples must be taken before any additional processing. Further processing, handling and distribution will introduce variables that will interfere with the interpretation of the data intended to describe slaughter and dressing processes. For this reason, carcasses were sampled after chilling, the end point in slaughter and dressing. For those carcasses that were either hot boned or shipped prior to chilling, sampling occurred after the final wash prior to hot boning or shipping.

Carcass Sample Sites:

The flank, brisket, and rump were chosen for this program because these locations are most likely to become contaminated during the slaughter/dressing procedure. If only hide-on calves were available for sampling, then sponge sampling was performed inside the visceral cavity at the flank and brisket, and inside the pelvic area at the rump. Hocks and shanks are other good locations. However, these sites did not provide the large surface area necessary for sponge sampling.

Sample Collection and Description:

Samples were aseptically collected by FSIS Inspectors-in-Charge following the procedures described in Appendices E and F of the PR/HACCP Regulation of July 25, 1996[(1)] (procedures subsequently described in FSIS Directive 10,230.5 [2/4/98]), and instructions provided on computer-generated sample request forms. A sterile sponge, hydrated with 10 ml of cold sterile Buffered Peptone Water (BPW) was used to swab, within a sterile 10x10 cm, plastic template, a 300 cm^2 surface area composite. The composite included one flank site (100 cm^2), one brisket site (100 cm^2), and one rump site (100 cm^2). One sponge sample was collected from one cattle carcass half for *Salmonella* analysis, and a separate sponge sample was collected from a second cattle carcass half for generic *E. coli* analysis at the same sampling period. The individually bagged, sponge samples were then placed in an insulated shipper with chilled gel-ice packs capable of maintaining refrigeration temperatures and shipped the same day as sample collection to the designated laboratory via an overnight delivery service. Samples were collected Monday through Friday during slaughter operations. Samples collected and shipped on Fridays were labeled specifying "For Saturday Delivery" on the shipping box. Only samples received at the laboratory the day after sample collection, with a sample receipt temperature of 0 to 10EC (inclusive), were analyzed. Samples received outside of those constraints were discarded. Only one analysis, either for *Salmonella* or generic *E. coli*, was performed on each individual sponge of the paired sponge samples.

Selection of Organisms:

A discussion of the choice of organisms to be used in establishing microbiological guidelines is found in the study entitled "An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients" published by the Subcommittee on Microbiological Criteria of the National Research Council, National Academy of Sciences[(10)]. That rationale was reviewed and assessed for applicability and incorporation into this program as it was also used in previous FSIS baseline sampling programs.

For the purposes of the cattle sponge sampling program, two microorganisms were selected for analysis. One was selected from a group of organisms most

often associated with human illness as determined by foodborne illness reports[11][12] or certain pathogens of concern because of the severity of the illness they produce in humans:

- *Salmonella*

The other organism was selected from groups of bacteria that are thought to be of value as indicators of general hygiene or process control.

- Generic *Escherichia coli*

Analytical Methods:

To the sponge designated for *Salmonella* analysis, an additional 50 ml BPW was added to bring the total volume to 60 ml. This sponge with BPW was stomached in the original bag for two minutes. The procedure used for qualitative *Salmonella* analysis was the same as that described in Appendix E of the July 25, 1996 PR/HACCP Regulation[1].

To the sponge designated for generic *E. coli* analysis, an additional 15 ml BPW was added to bring the total volume to 25 ml. This sponge with BPW was stomached in the original bag for two minutes and then analyzed according to the procedure for quantitative, generic *E. coli* analysis described in Chapter 3, Section 3.5c of the Microbiology Laboratory Guidebook[13] employing rehydratable Petrifilm™. Appropriate dilutions were made to obtain an end point and Petrifilm™ was inoculated in duplicate for each dilution. After determining the average Petrifilm™ count, it was multiplied by the appropriate dilution factor and then divided by 12 to obtain the count on a cfu/cm^2 basis.

RESULTS

The results of this sponge sampling microbiological baseline data collection program for cattle carcasses, are presented in the tables found in this report. Table 1 presents the prevalence, or frequency of occurrence, of the selected microorganisms from slaughtered cattle carcass surfaces. An estimated national prevalence of 16.6% was found for generic *E. coli* in 1,881 sampled carcasses and a corresponding national prevalence of 1.2% was found for *Salmonella* in 1,881 sampled carcasses.

Table 2 presents the mean level of generic *E. coli* quantitatively recovered from cattle carcasses that tested positive for this organism. The mean level in this table is expressed as both the $\log_{10}$ mean and the geometric mean; the geometric mean is the antilog of the $\log_{10}$ mean. The geometric mean of viable

generic *E. coli* recovered from sampled cattle carcasses in this program was 0.26 colony forming units per square centimeter (cfu/cm^2).

Table 3 shows the frequency with which the positive samples enumerated for generic *E. coli* fall within the specified ranges. All of the 1,881 cattle carcasses analyzed had 100 or fewer and 98.9% had 10 or fewer cfu/cm^2 generic *E. coli* on their surfaces.

DISCUSSION

This report presents the primary goal of the program: an updated, limited microbial profile of cattle carcasses, as produced under Federal inspection, in regard to the prevalence of *Salmonella* and the prevalence and quantitation of generic *E. coli* as determined by non-destructive sponge sampling.

The basic findings of this baseline program, limited to two surveyed microorganisms, revealed a low prevalence of both *Salmonella* (1.2%) and generic *E. coli* (16.6%) with a low mean number of generic *E. coli* on the carcass surface (0.26 cfu/cm^2). The data provided from this baseline study are very useful for establishment of generic *E. coli* and *Salmonella* guidelines in slaughtered cattle using the sponge sampling technique.

The presence of pathogenic bacteria on the surface of cattle carcasses, even though of low prevalence, emphasizes the need for proper refrigeration, handling, and cooking of beef products throughout the food chain. In addition, special care must also be taken to avoid cross contamination of other ready-to-eat food products with raw beef products and in the cleaning and disinfection of food preparation work surfaces after handling raw beef products. However, the prevalence of *Salmonella* and levels of generic *E. coli* enumerated on cattle carcasses suggest that recommended cooking temperatures would render products produced from these carcasses safe, as long as the carcasses and the products produced from them, are maintained at refrigeration temperatures throughout subsequent distribution, storage, processing, marketing and preparation for consumption.

Table 1. Prevalence of Generic *Escherichia coli* and *Salmonella* from Cattle Carcass Sponge Samples

Microorganism	Samples Analyzed	Number Positive	Prevalence	SE[1]
INDICATOR ORGANISM				
Generic *Escherichia coli*	1881	312	16.6	0.9
PATHOGENIC ORGANISM				
Salmonella	1881	23	1.2	0.3

[1] Standard error of prevalence using the binomial distribution.

Source: Nationwide Sponge Microbiological Baseline Data Collection Program: Cattle (June 1997 - May 1998)

Table 2. Mean Level of Generic *Escherichia coli* (per cm^2) from Cattle Carcass Sponge Samples

Microorganism	Number of Samples Quantified	Number of Samples Positive	Levels of Positives[1]			
			Log_{10}		Geometric Mean	
			Mean	SE[2]	Mean	95% CI[3]
INDICATOR ORGANISM						
Generic *Escherichia coli*	1881	312	- 0.58	0.04	0.26	(0.22, 0.32)

[1] Includes only positive samples.

[2] Standard error of the mean log of positive samples.

[3] Confidence Interval.

Source: Nationwide Sponge Microbiological Baseline Data Collection Program: Cattle (June 1997 - May 1998)

Table 3. Generic *Escherichia coli* Distribution (per cm^2) from Cattle Carcass Sponge Samples

Range, cfu/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
0 [1]	1569	83.4	1569	83.4
0.01 - 1	230	12.2	1799	95.6
1.01 - 10	62	3.3	1861	98.9
10.01 - 100	20	1.1	1881	100.0
TOTALS	1881	100.0	-	-

[1] Negative by method (Limit of detection = 0.042 cfu/cm^2).

Source: Nationwide Sponge Microbiological Baseline Data Collection Program: Cattle (June 1997 - May 1998)

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