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Nationwide Beef Microbiological Baseline Data Collection Program: Cows and Bulls

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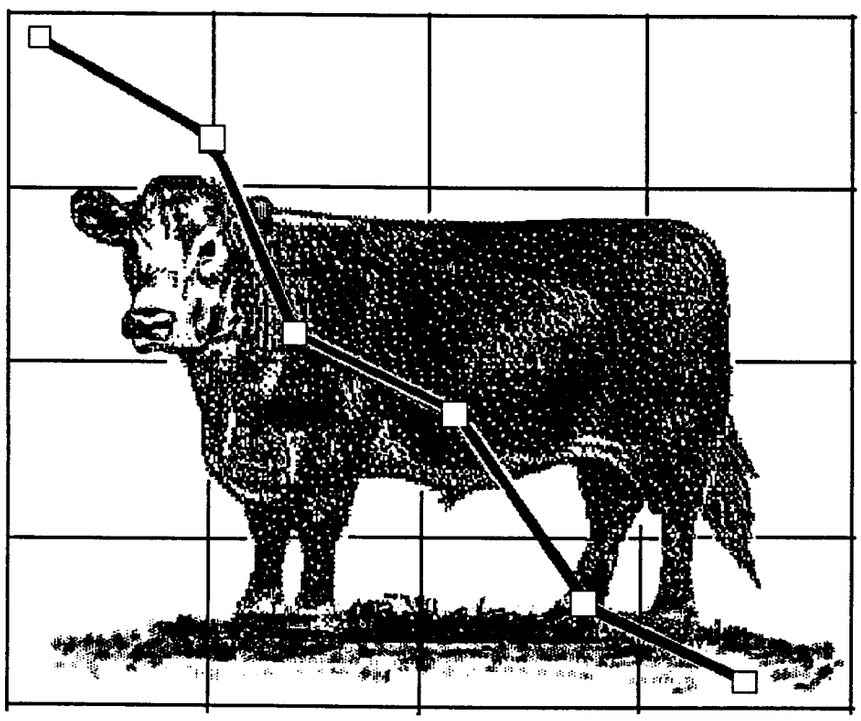

FOREWORD

This publication is a compilation of data obtained from the Nationwide Beef Microbiological Baseline Data Collection Program for Cows and Bulls for the period from December 1993 through November 1994. The program was initiated to estimate the prevalence and levels of bacteria of public health concern on cow and bull carcasses as currently produced. The program was designed through consultation with various staffs in the Agency and advice from scientists and organizations outside the Agency. The Microbiology Division in conjunction with the Statistics and Data Systems Division coordinated the conduct of the program, provided data analysis and prepared this report. The microbiological analyses were conducted by the Technical Support Laboratories located in Athens, GA, St. Louis, MO, and Alameda, CA. Sample collection was the responsibility of the FSIS Inspectors-in-Charge without whom this program could not have been accomplished.

NATIONWIDE BEEF MICROBIOLOGICAL BASELINE DATA COLLECTION PROGRAM: COWS AND BULLS DECEMBER 1993 - NOVEMBER 1994

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EXECUTIVE SUMMARY

From December 1993 through November 1994, tissue samples from 2,112 cow or bull carcasses were collected from establishments operating under Federal inspection. These samples were collected to estimate the prevalence and levels of bacteria of public health concern on cow and bull carcasses as currently produced. The establishments in the program are responsible for approximately 99% of all cows and bulls slaughtered and 18% of total beef animals slaughtered under Federal inspection. The tissue samples were analyzed for the presence of those bacteria most often associated with human illness as determined by foodborne illness reports, other pathogens of interest because of the severity of human illness they produce, and certain bacteria, or groups of bacteria, thought to be of value as indicators of general hygiene or process control. *Clostridium perfringens* was recovered from 8.3% of 2,112 carcasses, *Staphylococcus aureus* was recovered from 8.4% of 2,112 carcasses, *Listeria monocytogenes* was recovered from 11.3% of 2,112 carcasses, *Campylobacter jejuni/coli* was recovered from 1.1% of 2,109 carcasses, *Salmonella* was recovered from 2.7% of 2,112 carcasses, and *Escherichia coli* O157:H7 was not recovered from any of the 2,112 carcasses. Aerobic plate counts (APC @35°C) of 100,000 or fewer colony forming units (cfu) per cm^2 were found in 96.3% of the samples, 92.2% contained 100 or fewer coliforms per cm^2, and 91.8% contained 10 or fewer *Escherichia coli* (Biotype I) per cm^2. Biotype I *E. coli* are generally considered non-pathogenic. The APC levels are in agreement with those reported by the National Academy of Sciences in 1985 as normal for freshly dressed beef carcasses in the United States[1].

INTRODUCTION

The Food Safety and Inspection Service (FSIS) is the Federal agency responsible for enforcing the Federal Meat Inspection Act and the Poultry 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 Acts primarily focus on the detection of diseased animals going to slaughter and on their rejection for use in 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 remove most of these bacteria, including many pathogens, but not all. 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, must be assumed to be present on the final raw product. This is a fact that has long been recognized by the Agency and by scientific experts around the world.

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.

The Agency designed this program to estimate the prevalence and levels of bacteria of public health concern on cow and bull carcasses as currently produced under Federal inspection.

OBJECTIVES

This non-regulatory program has two objectives:

1. To collect data which provide a general microbiological profile of cow and bull carcasses for selected microorganisms of various degrees of public health concern.

2. 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 Beef Microbiological Baseline Data Collection Program was designed to provide a microbiological baseline for cattle production. Cows and bulls were chosen as the target population for two key reasons: they constitute approximately 18 percent of beef animals slaughtered, and they are major source of raw materials for ground beef produced at the federal level. Steers and heifers, which constitute approximately 80% of beef animals

slaughtered were the subject of an earlier study.[2] The data obtained will enable the Agency to describe a microbiological profile of cow and bull carcasses produced under Federal inspection and to document changes in the profile over time. Additional applications of this approach have included similar programs aimed at describing general profiles of bacteria on steer and heifer carcasses, poultry and swine.

PROGRAM DESIGN

Plants Included in the Sampling Frame:

All establishments that slaughter an average of approximately 15 or more cows and/or bulls per week (approximately 780 or more per year) were included in the sample frame. There are approximately 185 establishments in this category. These establishments account for more than 99 percent of all cows and bulls slaughtered annually in federally inspected plants and constitute approximately 18% of domestic origin beef. The smallest establishments were excluded from the sampling frame since they account for less than 1% of all cows and bulls slaughtered and these plants may slaughter only intermittently. Including these establishments would add significant logistical difficulty without gaining appreciable additional information.

Sampling Design:

There were many factors that were considered in designing this sampling program. Among these were the size and variability of the population, the nature and number of bacteria to be investigated, the practical costs of sampling, competing program demands, and the type of information sought.

Both sampling and non-sampling errors can affect the reliability of results and, thus, 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 several sources inherent to the collection of samples, laboratory analysis and processing of data. Both types of errors were considered in determining the sample size.

A random sample of 3,080 carcasses was requested during the time frame of the study (approximately 57 carcasses per week). Of these, laboratory results were obtained for 2,112 carcasses. Some samples may not have been collected for various reasons, such as the plant did not slaughter that particular week, and other samples may not have been analyzed if, for example, they were received either too warm or too cold (outside the constraints / limitations of the study). Over 19,000 laboratory analyses were subsequently conducted.

A sample size of about 2,100 carcasses ensures reasonable levels of precision for yearly estimates given the expected prevalence of the bacteria included in this study. For example, as shown in Table 1, the estimated annual incidence rate for *Salmonella* is 2.7 percent with a standard error of 0.4 percent. The rate of 2.7% has a margin of error (with 95% confidence) of ±0.78% (1.96 x 0.4). Therefore, a 95% confidence interval for the annual incidence rate for *Salmonella* in the entire population of cows and bulls is 1.92 to 3.48 percent.

Establishments were randomly selected weekly using probabilities proportional to slaughter. Therefore, establishments slaughtering the largest number of cows and/or bulls were sampled more often than smaller establishments. Each time an establishment was selected, 3 surface samples from one randomly selected carcass half was requested. Due to the constraints imposed by the service for overnight delivery of samples to the laboratories, the random selection of the carcasses was restricted to the first shift, Monday through Thursday.

Data Limitations:

The program was designed to provide estimates of national prevalences and levels of selected microorganisms on cow and bull carcasses. The data obtained provides an indication of which microorganisms might be present on federally-inspected cow/bull carcasses.

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

Sampling Location Within the Plant:

To accomplish the objectives of this program, data must be derived from a significant point in the production process. A key factor in the microbial profile of beef is 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 have been sampled after chilling, the end point in slaughter and dressing.

There are good arguments for any number of plant sampling sites[3] [4] [5]. For the purposes of this program, the Agency chose to sample carcasses after chilling.

The cooler is appropriate for establishing a baseline of microbiological information describing the microbial profile of beef moving into commerce from Federally inspected

plants as it is the end point in the slaughter and dressing process. The cooler is also a point where the carcass comes to rest, allowing a window in time in which a sample can be taken.

Carcass Sample Sites:

The rump, brisket, and flank were chosen for this program because these locations are most likely to become contaminated during the slaughter/dressing procedure. Hocks and shanks are other good locations. However, these sites did not provide the large surface area necessary for sampling.

Sample Collection and Description:

Samples were collected by FSIS Inspectors-in-Charge following the procedures in FSIS Directive 10230.2 (8/6/92), instructions provided on computer generated sample collection request forms, and specific instructions applicable to this program. Samples consisted of subsamples taken from the rump, flank and brisket of a randomly selected carcass half that had cooled in the cooler for at least 12 hours. Each subsample consisted of a surface tissue section approximately 1 centimeter (0.5 inches) deep and comprising about 300 square centimeters (about 6 inches by 8 inches). Subsamples were separately bagged. The bags were placed in an insulated shipper with gel packs capable of maintaining refrigeration temperatures and shipped to the designated laboratory via an overnight delivery service. Only those samples received at the laboratory the day after sample collection, with a sample receipt temperature of 0°C to 10°C (inclusive) were analyzed. Those samples received outside those constraints were discarded.

Selection of Organisms:

A discussion of the choice of organisms to be used in microbiological criteria 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 for Foods and Food Ingredients of the National Research Council, National Academy of Sciences[1]. The rationale used in that book was reviewed and assessed for incorporation in this program.

For the purposes of this program, the organisms selected were those most often associated with human illness as determined by foodborne illness reports[6] or certain pathogens of concern because of the severity of the illness they produce in humans:

- *Salmonella*
- *Staphylococcus aureus* (coagulase positive staphylococci)
- *Clostridium perfringens*
- *Escherichia coli* O157:H7
- *Campylobacter jejuni/coli*
- *Listeria monocytogenes*

Data on certain bacteria, or groups of bacteria, thought to be of value as indicators of general hygiene or process control were also collected:

- Total coliforms
- *Escherichia coli* (Biotype I)
- Aerobic Plate Count (APC) at 35°C (total viable aerobic microorganisms)

Analytical Methods:

An analytical sample consisted of a composite of the individual flank, brisket and rump tissues taken from a cow or bull carcass for a total composite area of of 60 cm^2.

The laboratory methods used were identical to those used in the first in the series of the Nationwide Data Collection Programs, Steers and Heifers.[2]

The Aerobic Plate Count (APC) at 35°C, Total coliforms, *E. coli* (Biotype I), *C. perfringens* and *S. aureus* are reported as colony forming units (cfu) per square centimeter (cm^2) of surface area analyzed. *L. monocytogenes*, *C. jejuni/coli*, *E. coli* O157:H7 and *Salmonella*, because they require enrichment, are reported as the Most Probable Number estimate of bacterial population density (MPN) per square centimeter of surface area analyzed. For these pathogenic bacteria, samples were first analyzed by a qualitative enrichment method with a minimum detection level of 0.02 organisms per cm^2 in a 60 cm^2 sample. If positive, the analysis was repeated on a separate portion of the original sample composite using the MPN method for enumeration which has a minimum detection level of 0.03 organism MPN/cm^2. In some cases, insufficient tissue was available to perform all required enumeration analyses. Differences in the number of samples enumerated are noted in the data tables.

RESULTS

The results are presented in tables and figures found in this report. Table 1 and Figure 1 present the prevalence, or frequency of occurrence, of the selected microorganisms on the surfaces of the raw beef carcasses sampled. Table 2 and Figure 2 present the mean level of selected microorganisms recovered from the surfaces of the raw beef carcass surfaces that tested positive for the particular microorganism. The mean levels in Table 2 are expressed as both the log mean and the geometric mean; the geometric mean is the antilog of the log mean. For example, in Table 2, the geometric mean level of viable aerobic microorganisms recovered in the Aerobic Plate Count @35°C was approximately 1,130 cfu per square centimeter. Tables 3 - 7 and Figures 3 - 7 show the frequency within which all samples enumerated for each microorganism or group of microorganisms fall within specified intervals. Tables 8 - 10 and Figures 8 - 10 show the frequency within which only

the positive samples enumerated for each microorganism fall within specified intervals. Following is a brief summary of the results.

Viable aerobic bacteria (Aerobic Plate Count @35°C) were recovered from the surface of 99.6% of the 2,112 carcasses tested in this program (Table 1, Figure 1). Coliforms were recovered from 32.4% of 2,112 carcasses and *E. coli* (Biotype I) was recovered from 15.8% of 2,112 carcasses. *C. perfringens* was recovered from 8.3% of 2,112 carcasses, *S. aureus* was recovered from 8.4% of 2,112 carcasses, *L. monocytogenes* was recovered from 11.3% of 2,112 carcasses, *C. jejuni/coli* was recovered from 1.1% of 2,109 carcasses, and *Salmonella* was recovered from 2.7% of 2,112 carcasses. *E. coli* O157:H7 was not recovered from any of the 2,112 carcasses.

On carcasses that tested positive, the geometric mean of the Aerobic Plate Count @35°C was 1,130 cfu/cm^2 (Table 2, Figure 2), the geometric mean of coliforms was 40 cfu/cm^2 and the geometric mean of *E. coli* (Biotype I) was 33 cfu/cm^2. When positive for a specific pathogen, the geometric mean on carcasses was: 47 *C. perfringens* cfu/cm^2; 25 *S. aureus* cfu/cm^2; 0.3 *L. monocytogenes* MPN/cm^2; 0.1 *C. jejuni/coli* MPN/cm^2; and 0.3 *Salmonella* MPN/cm^2.

Of the samples tested for indicator organisms (Tables 3-5, Figures 3-5), 96.3% had aerobic plate counts (APC @35°C) of 100,000 or less colony forming units (cfu) per cm^2, 92.2% contained 100 or fewer coliforms per cm^2, and 91.8% contained 10 or fewer *E. coli* (Biotype I) per cm^2. Biotype I *E. coli* are generally considered to be non-pathogenic.

The highest level detected (Tables 6-10, Figures 6-10) for each of the various pathogens was: less than 100,000 cfu/cm^2 for *C. perfringens*; less than 10,000 cfu/cm^2 for *S. aureus*; 43.0 MPN/cm^2 for *L. monocytogenes*; less than 0.3 MPN/cm^2 for *C. jejuni/coli*; and 240 MPN/cm^2 for *Salmonella*.

Six pathogens, *S. aureus*, *C. perfringens*, *L. monocytogenes*, *Salmonella*, *E. coli* O157:H7, and *C. jejuni/coli* were not recovered from 1,538 (72.8%) of the 2,112 carcasses tested (Table 11, Figure 11). Four hundred eighty-four carcasses contained only one pathogenic bacterial species, whereas 82 carcasses contained two species and only 8 carcasses contained a total of three species. No carcasses tested contained more than 3 pathogenic species.

DISCUSSION

This manuscript presents the primary goal of this program: a microbial profile of cow and bull carcasses which includes the number and types of bacteria recovered. The presence of pathogenic bacteria on the surfaces of carcasses, even though infrequent, 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 ready-to-eat foods with raw beef products and in the cleaning and disinfection of food preparation work surfaces after handling raw beef products.

Current procedures in use in federally inspected establishments are generally unable to completely remove viable bacteria from cow or bull carcasses during slaughter and dressing operations. APC levels recovered in this study (Figure 3), however, agree with historical data reported in 1985 by the National Academy of Sciences for freshly dressed beef carcasses in the United States[1] in which APCs were found to range normally around 100 to 10,000 cfu/cm^2. This study also showed that pathogenic bacteria cited as causing foodborne illness by the CDC can be isolated from the surfaces of carcasses after slaughter and dressing operations. However, the highest levels of pathogenic bacteria enumerated were at levels where recommended cooking temperatures would render product 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.

The results from this study may be useful for comparison purposes, for example, for comparing these results with future survey results obtained using the same methodology and for performing various hazard analyses of the slaughter process.

TABLES

Table 1. Prevalence of Selected Microorganisms on Raw Beef Carcass Surface Samples

Microorganism	Samples Analyzed	Samples Positive		
		Number Positive[1]	Percent Positive	SE [2]
INDICATOR ORGANISMS				
Aerobic Plate Count @ 35°C	2,112	2,104	99.6	0.1
Total Coliforms	2,112	684	32.4	1.0
Escherichia coli (Biotype I)	2,112	333	15.8	0.8
PATHOGENIC ORGANISMS				
Clostridium perfringens	2,112	176	8.3	0.6
Staphylococcus aureus	2,112	178	8.4	0.6
Listeria monocytogenes	2,112	238	11.3	0.7
Campylobacter jejuni/coli	2,109[3]	24	1.1	0.2
Escherichia coli O157:H7	2,112	0	0.0	NA[4]
Salmonella	2,112	56	2.7	0.4

[1] Positive by qualitative method.

[2] Standard Error using the binomial distribution.

[3] Insufficient tissue available to perform all analyses.

[4] NA= Not applicable

Source: Nationwide Beef Microbiological Baseline Data Collection Program: Cows and Bulls (December 1993 - November 1994).

Table 2. Mean Level of Selected Microorganisms per Square Centimeter on Raw Beef Carcass Surface Samples

Microorganism	Number of Samples Quantified[1]	Number of Samples Positive[2]	Level of Positives			
			Log_{10} Mean		Geometric Mean	
			Mean [3]	SE [4]	Mean [3]	95% CI [5]
INDICATOR ORGANISMS						
Aerobic Plate Count @ 35°C	2,112	2,104	3.05	0.02	1,130	(1,030, 1,240)
Total Coliforms	2,112	684	1.60	0.04	40	(34, 46)
Escherichia coli (Biotype I)	2,112	333	1.52	0.05	33	(26, 42)
PATHOGENIC ORGANISMS						
Clostridium perfringens	2,112	176	1.67	0.06	47	(35.7, 61.3)
Staphylococcus aureus	2,112	178	1.39	0.04	25	(20.6, 29.9)
Listeria monocytogenes	231	109	- 0.55	0.07	0.3	(0.2, 0.4)
Campylobacter jejuni/coli	16[6]	3	- 1.96	0.14	0.1	(0.1, 0.2)
Escherichia coli O157:H7	0	NA[7]	NA[7]	NA[7]	NA[7]	NA[7]
Salmonella	53[6]	21	- 0.57	0.24	0.3	(0.1, 0.8)

1 Positive by qualitative method, subsequently analyzed by quantitative method.
2 Positive by quantitative method.
3 Level only of those samples found positive by quantitative method.
4 Standard error of the mean of positive samples.
5 Confidence Interval.
6 Insufficient tissue available to perform all analyses.
7 NA= Not applicable

Source: Nationwide Beef Microbiological Baseline Data Collection Program: Cows and Bulls (December 1993 - November 1994).

Table 3. Aerobic Plate Count @35°C Distribution on Raw Beef Carcass Surface Samples

Range, cfu/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
<1 [1]	8	0.4	8	0.4
1 - 10	13	0.6	21	1.0
11 - 100	261	12.4	282	13.4
101 - 1,000	856	40.5	1,138	53.9
1,001 -10,000	651	30.8	1,789	84.7
10,001 - 100,000	244	11.6	2,033	96.3
100,001 - 1,000,000	68	3.2	2,101	99.5
1,000,001 - 10,000,000	9	0.4	2,110	99.9
>10,000,000	2	0.1	2,112	100.0
TOTAL	2,112	100.0	-	-

[1] Negative by method.

Source: Nationwide Beef Microbiological Baseline Data Collection Program: Cows and Bulls (December 1993 - November 1994).

Table 4. Total Coliform Distribution on Raw Beef Carcass Surface Samples

Range, cfu/cm^2	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
<1 [1]	1,428	67.6	1,428	67.6
1 - 10	277	13.1	1,705	80.7
11 - 100	242	11.5	1,947	92.2
101 - 1,000	109	5.2	2,056	97.3
1,001 -10,000	35	1.7	2,091	99.0
10,001 - 100,000	16	0.8	2,107	99.8
100,001 - 1,000,000	5	0.2	2,112	100.0
TOTAL	2,112	100.0	-	-

[1] Negative by method.

Source: Nationwide Beef Microbiological Baseline Data Collection Program: Cows and Bulls (December 1993 - November 1994).