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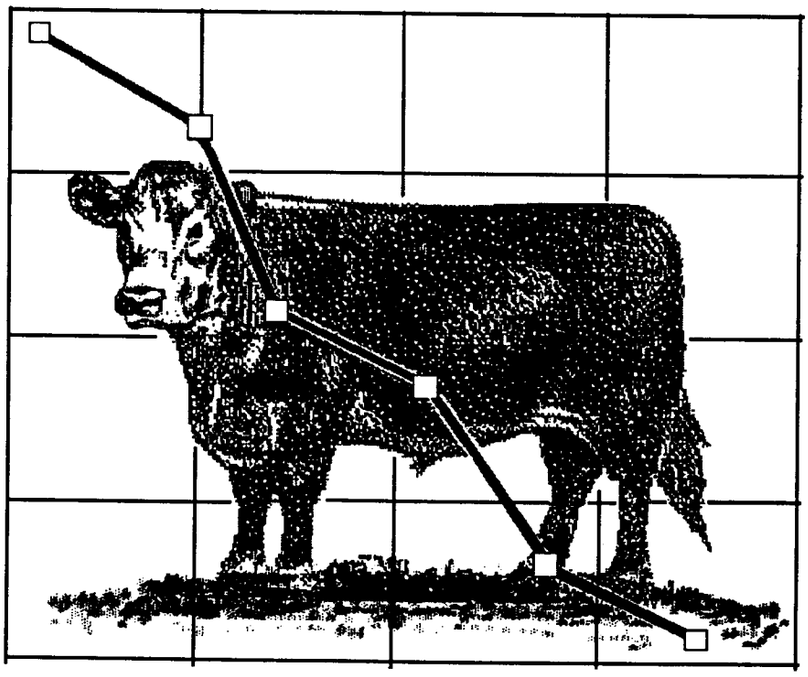

FOREWORD

This publication is a compilation of data obtained from the Nationwide Beef Microbiological Baseline Data Collection Program for Steers and Heifers for the period from October 1992 through September 1993. The program was initiated to define the prevalence and levels of bacteria of public health concern on steer and heifer carcasses as currently produced. The program was designed through consultation with various staffs in the Agency and advice from many individuals 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 Laboratory Divisions 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: STEERS AND HEIFERS OCTOBER 1992 - SEPTEMBER 1993

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EXECUTIVE SUMMARY

From October 1992 through September 1993, tissue samples representing approximately 2,100 steer or heifer 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 steer and heifer carcasses as currently produced. The establishments included in the program are responsible for approximately 99% of domestic origin steer and heifer production. 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 2.6% of 2,079 carcasses, *Staphylococcus aureus* was recovered from 4.2% of 2,089 carcasses, *Listeria monocytogenes* was recovered from 4.1% of 2,089 carcasses, *Campylobacter jejuni/coli* was recovered from 4.0% of 2,064 carcasses, *Escherichia coli* O157:H7 was recovered from 0.2% of 2,081 carcasses and *Salmonella* was recovered from 1.0% of 2,089 carcasses. Of the samples tested, 93.1% had aerobic plate counts (APC @35°C) of 10,000 or fewer colony forming units (cfu) per cm^2, 96.4% contained 100 or fewer coliforms per cm^2, and 95.9% 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 as normal in 1985 by the National Academy of Sciences for freshly dressed beef carcasses in the United States[1].

INTRODUCTION

During the period 1973-1987, 1,879 foodborne disease outbreaks associated with bacterial agents involving 108,906 cases were reported to the Centers for Disease Control and Prevention (CDC)[2]. The most frequently identified bacterial agent was *Salmonella* followed in order by *Staphylococcus aureus*, *Clostridium botulinum*, *Clostridium perfringens* and *Shigella*. Food products of all types were identified as vehicles including beef, pork, turkey and chicken products. The bacterial agents most frequently identified for illnesses associated with meat and poultry products were *Salmonella*, *S. aureus*, *C. perfringens* and pathogenic *Escherichia coli* including

E. coli O157:H7. For these bacteria, in particular, the most frequently reported factors identified as contributing to the outbreaks were improper holding temperatures and inadequate cooking. Outbreaks were associated with food prepared in homes, delicatessens, cafeterias, restaurants, schools, at picnics, churches, and camps. Overall, meat and poultry products accounted for 23.3% of reported bacterial foodborne outbreaks. Beef accounted for 8.5% of these outbreaks and may be more frequently involved in sporadic cases of foodborne illness.

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 many 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.

USDA is currently investing millions of dollars in food safety research to develop animal husbandry practices that will be less conducive to pathogen growth in live animals, to develop better farm control practices which reduce the occurrence of pathogens on farms, to develop better farm animal hauling practices which reduce cross contamination and to develop better slaughter and processing procedures which remove even more bacteria. Even a small contribution in any one of these areas can help to reduce further the occurrence of pathogenic bacteria in raw meat and poultry products.

Bacteria that produce toxins such as *S. aureus* and *C. perfringens* are, generally, not considered a hazard unless the level of these bacteria in a product is greater than 1,000,000 organisms/gram [1]. Generally, reaching these levels requires prolonged periods of temperature abuse in the range of 45°F to 130°F either before or after cooking, as is usually the case in staphylococcal intoxication, or after

cooking, as is usually the case in *C. perfringens* gastroenteritis. The level of bacteria such as *Salmonella*, pathogenic *E. coli*, *Campylobacter jejuni/coli* or *Listeria monocytogenes* in a product necessary to cause illness is not as well defined. It is generally accepted that for most individuals, high levels of pathogens are necessary; however, this may not be the case for individuals with weakened immune systems, older individuals, very young individuals and pregnant women. For this reason, ready-to-eat products prepared for human consumption are generally not considered safe if found to contain pathogenic organisms. Since these bacteria are readily killed by cooking, proper cooking of products that have been carefully handled will eliminate these bacteria.

FSIS has prescribed cooking procedures for certain ready-to-eat meat or poultry products that have been implicated in human illness. These cooking procedures must be used when these products are prepared in plants under federal inspection. The cooking procedures have been shown through research to be effective in eliminating the vegetative pathogens which must be assumed to be present in raw meat and poultry[3] [4] [5] [6] [7]. The use of these cooking procedures, together with sanitary handling, proper refrigeration or freezing, and strict separation of cooked products from raw products from the time of cooking until the product is packaged for sale, provide a high degree of confidence that the product is safe.

FSIS has conducted microbiological monitoring programs for a number of years that are designed to encourage the industry to continually monitor its procedures for the production of cooked, ready-to-eat meat and poultry products to assure they meet regulatory requirements. These programs have concentrated on the detection of *Salmonella* and *L. monocytogenes* in packaged cooked product and, together with the prescribed regulations and the actions taken by the industry, have been recognized by investigators at the CDC as effective in reducing consumer risk from both salmonellosis and listeriosis[8] [9].

Still, most of these precooked, "ready-to-eat" or "heat and eat" products must be maintained under strict temperature control (either refrigerated or frozen) throughout distribution and sale and home storage to assure continued safety. These products also have a finite shelflife beyond which products should not be used.

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, they can cause foodborne illness.

The Agency designed this program to estimate the prevalence and levels of bacteria of public health concern on steer and heifer carcasses as currently produced.

OBJECTIVES

This non-regulatory program has two objectives:

1. To collect data which provide a general microbiological profile of fed cattle (steer and heifer) 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 fed cattle production. Fed cattle were chosen as the target population for two key reasons: they constitute approximately 80 percent of beef animals slaughtered, and they are the immediate source of most retail cuts. The data obtained will enable the Agency to describe a microbiological profile of fed cattle produced under Federal inspection and to document changes in the profile over time. Subsequent applications of this approach will include similar programs aimed at describing general profiles of bacteria on poultry and swine. The second objective will lead to further, more specialized studies that will evaluate specific products or the effect of specific decontamination intervention strategies.

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.

PROGRAM DESIGN

Plants Included in the Sample Frame:

All establishments that slaughter an average of approximately 40 or more fed cattle per week (2,100 or more per year) were included in the sample frame. There are approximately 100 establishments in this category. These establishments account for more than 99 percent of all fed cattle slaughtered in federally inspected plants and approximately 80% of domestic origin beef. Sampling at the 100 percent level was not chosen because many of the additional plants are very small and slaughter only intermittently. This 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 2,829 carcasses was requested during the 1993 fiscal year (over 54 carcasses per week). Of these, laboratory results were obtained for 2,089 carcasses. Over 18,000 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 *C. jejuni/coli* is 4.0 percent with a standard error of 0.43 percent. The rate of 4.0% has a margin of error (with 95% confidence) of $\pm 0.84\%$ (1.96 x 0.43). Therefore, a 95% confidence interval for the annual incidence rate for *C. jejuni/coli* in the entire population of steers and heifers is 3.16 to 4.84 percent.

Establishments were randomly selected weekly using probabilities proportional to slaughter. Establishments slaughtering the largest number of fed cattle were sampled more often than smaller establishments.

Data Limitations:

The program was designed to provide national level estimates and distributions. Thus, the reliability of data with respect to individual plants would not be meaningful and the interpretation of individual plant data may be misleading.

Sampling Location Within the Plant:

There are good arguments for any number of plant sampling sites[10] [11] [12]. 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.

The Agency recognizes that current practices emphasize trimming external fat from beef cuts, which might alter the microbial profile of the cuts as purchased by consumers. However, the microbial profile of trimmed cuts could be the subject of future, more targeted studies. Products such as ground beef or boxed beef were deemed inappropriate for the goals of this program. Such products are further influenced by handling procedures during the boning and grinding operations and by growth and multiplication of bacteria under refrigeration.

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. Next day delivery was specified. Samples were refrigerated, not frozen.

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[2]. 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[1] 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 steer or heifer carcass.

Standard AOAC methods were chosen for analysis of coliforms, *E.coli* (Biotype I), *S. aureus*, and total aerobic plate count. However, since consensus on testing methods for the recovery of the other organisms from meat and poultry has not been reached, the methods chosen were those that have been proven effective at recovering these bacteria of public health concern from meat and poultry.

The method for the enumeration of *C. perfringens* is referenced in the Compendium of Methods for the Microbiological Examination of Foods.

The *L. monocytogenes* method chosen was the same FSIS method that is currently being used for the recovery of the organism from cooked, ready-to-eat meat and poultry products. The method has been published and successfully used worldwide[13] [14] [15]. It is generally recognized as one of the best methods available for recovery of the organism from meat and poultry products.

No collaboratively studied method is currently available for the recovery of *C. jejuni/coli*. The method chosen is the method currently considered the best available by researchers in that field of study.

The *E. coli* O157:H7 method chosen is an FSIS method considered by many researchers as one of the best methods currently available. It has been published[16] [17] [18] and is referenced in the Compendium of Methods for the Microbiological Examination of Foods.

The *Salmonella* benchmark method used in previous surveys conducted by FSIS was established in the late 1960s[19] and used since that time in response to the recommendation of a U.S. Advisory Committee on *Salmonella* to establish a benchmark, or a point of comparison, on which progress in the control of *Salmonella* could be judged. There have been many improvements in *Salmonella* methodology since these benchmark studies were initiated. This was the appropriate time to include these changes. Most notable among the changes is the change from Lactose Broth to Buffered Peptone Water. Buffered Peptone Water (BPW) has been recommended by the International Organization for Standardization (ISO) and the International Commission on Microbiological Specifications for Foods (ICMSF) for the non-selective first step in the isolation of salmonellae from foods. A second change is a Selenite Cystine enrichment step in addition to the standard TT Broth[20] enrichment.

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 475 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 - 11 and Figures 8 - 11 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 98.8% of the 2,089 carcasses tested in this program (Table 1, Figure 1). Coliform bacteria were recovered from 16.3% of 2,089 carcasses and *E. coli* (Biotype I) was recovered from 8.2% of 2,089 carcasses. *S. aureus* was recovered from 4.2% of 2,089 carcasses, *Salmonella* was recovered from 1.0% of 2,089 carcasses, *C. perfringens* was recovered from 2.6% of 2,079 carcasses, *C. jejuni/coli* was recovered from 4.0% of 2,064 carcasses, *L. monocytogenes* was recovered from 4.1% of 2,089 carcasses, and *E. coli* O157:H7 was recovered from 0.2% of 2,081 carcasses.

On carcasses that tested positive, the geometric mean of the Aerobic Plate Count @35°C was 474.7 cfu/cm^2 (Table 2, Figure 2), the geometric mean of coliforms was 35.3 cfu/cm^2 and the geometric mean of *E. coli* (Biotype I) was 35.3 cfu/cm^2. When positive for a specific pathogen, the geometric mean on carcasses was: 24.3 *S. aureus* cfu/cm^2; 0.1 *Salmonella* MPN/cm^2; 45.1 *C. perfringens* cfu/cm^2; 0.1 *C.*