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Nationwide Broiler Chicken Microbiological Baseline Data Collection Program

July 1994 - June 1995

FOREWORD

This publication is a compilation of data obtained from the Nationwide Broiler Chicken Microbiological Baseline Data Collection Program for the period July 1994 through June 1995. The program was initiated to estimate the prevalence and levels of bacteria of public health concern on broiler chicken 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 BROILER CHICKEN MICROBIOLOGICAL BASELINE DATA COLLECTION PROGRAM JULY 1994 - JUNE 1995

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EXECUTIVE SUMMARY

From July 1994 through June 1995, 1,297 broiler chicken carcasses were collected from establishments operating under Federal inspection. These carcasses were analyzed to estimate the prevalence and levels of bacteria of public health concern on broiler carcasses as currently produced. The establishments in the program are responsible for approximately 99% of all chickens slaughtered in the U.S. and approximately 94% of total poultry animals slaughtered under Federal inspection. The broiler carcasses 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 42.9% of the 1,297 broiler carcasses analyzed, *Staphylococcus aureus* was recovered from 64.0%, *Listeria monocytogenes* was recovered from 15.0%, *Campylobacter jejuni/coli* was recovered from 88.2%, and *Salmonella* was recovered from 20.0%. *Escherichia coli* O157:H7 was not recovered from any of the 1,297 broiler carcasses analyzed. Aerobic plate counts (APC @ 35°C) of 100,000 or fewer colony forming units per milliliter (cfu/ml) carcass rinse fluid were found in 99.5% of the samples, 97.4% contained 1,000 or fewer total coliform cfu/ml, and 98.0% contained 1,000 or fewer *Escherichia coli* (Biotype I) cfu/ml. When converted to cfu per square centimeter broiler carcass surface area (cfu/cm^2), aerobic plate counts of 100,000 or fewer cfu/cm^2 were found on 99.9% of the broiler carcasses, 99.2% contained 1,000 or fewer cfu/cm^2 total coliforms, and 99.4% contained 1,000 or fewer *E. coli* (Biotype I) cfu/cm^2. Biotype I *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 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 that 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 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 meat and poultry, 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 from normal, healthy broiler chickens can contain a variety of bacteria, including some pathogens. Refrigerated raw poultry 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 poultry is not properly cooked, held, cooled, and stored, the pathogens present could cause foodborne illness if the product is consumed.

OBJECTIVES

This non-regulatory program had two objectives:

1. To collect data that provide a general microbiological profile of broiler chickens 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 Broiler Chicken Microbiological Baseline Data Collection Program focused on establishing a microbiological baseline for broiler production. These results on the presence and quantity of selected microorganisms are expressed as a national average. Broiler chickens were chosen as the target population because they constitute approximately 94 percent of all poultry slaughtered. The data obtained provides a microbiological description or profile of broilers as currently produced under Federal inspection. This approach is similar to the FSIS Nationwide Microbiological Baseline Data Collection Programs for steers and heifers[1], cows and bulls[2], and market hogs[3].

PROGRAM DESIGN

Establishments Included in the Sampling Frame:

There are approximately 230 broiler slaughter establishments currently under Federal inspection. Of these, only those establishments that slaughter more than 100,000 broilers per year were included in the sampling frame. In this category were approximately 200 establishments, which accounted for more than 99.9 percent of all broilers slaughtered. The remaining establishments were not included in the frame because many were very small and slaughtered broilers only intermittently. Sampling these very small establishments would have added significant logistical difficulty without providing appreciable additional information.

Sample Design:

There were many factors 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 many sources inherent in the collection of samples, laboratory analysis and processing of data. Both types of errors were considered in determining the sample size.

It was determined that a sample size of about 1,200 samples would ensure reasonable levels of precision for yearly estimates given the expected prevalence for the bacteria included in this study. To achieve this number, a random sample of 1871 broiler carcasses was requested during the 52-week time frame of the study (approximately 36 per week). Of these, laboratory results were obtained for 1297 broiler carcasses. Some samples were not collected for various reasons, such as the establishment did not slaughter that particular week. Other samples were collected but not analyzed if, for example, they were received either too warm or too cold (outside the constraints/limitations of the study).

Establishments were randomly selected weekly using probabilities proportional to slaughter. Therefore, establishments slaughtering the largest number of broiler chickens were sampled more often than smaller establishments. Due to the constraints imposed by the service contracted 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 broiler carcasses. The data obtained provide an indication of which microorganisms might be present on federally inspected broiler 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.

Sampling Location Within the Establishment:

To accomplish the objectives of this program, data must be derived from a significant point in the production process. Key factors in the microbial profile of broiler chickens are the slaughter and evisceration processes conducted under Federal inspection. To evaluate these processes, sample carcasses 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 evisceration processes. For this reason, carcasses were sampled from the drip line after the chill tank, the end point in slaughter and evisceration.

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. A sample consisted of one randomly selected whole broiler carcass, aseptically placed into a sterile bag, closed securely and double bagged. The double-bagged sample was then 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 not analyzed.

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[4] . 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[5] [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:

The analytical sample for this study consisted of the rinse fluid recovered after shaking the broiler carcass in 400 ml of sterile Butterfield's Phosphate Diluent[7]. The subsequent laboratory methods used to analyze the carcass rinse fluid were identical to those used in all prior and subsequent Nationwide Microbiological Baseline Data Collection Programs.

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 milliliter (ml) of carcass rinse fluid analyzed. *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella*, because they require enrichment, are reported as the Most Probable Number estimate of bacterial population density per ml (MPN/ml) of carcass rinse fluid analyzed. For these pathogenic bacteria, samples were first analyzed by a qualitative enrichment method with a minimum detection level of 0.03 organisms per ml. If positive, the analysis was repeated on a separate portion of the original carcass rinse fluid using the MPN method for enumeration that has a minimum detection level of 0.03 organism MPN/ml. Reserved carcass rinse fluid was frozen with 15% glycerin as a cryopreservative, therefore the MPN enumeration results for *L. monocytogenes* and *Salmonella* have been adjusted to account for the additional dilution. For one sample positive for *L. monocytogenes*, insufficient reserve carcass rinse fluid was available to perform the enumeration analysis, as noted in Tables 2 and 2a. All samples were analyzed for *C. jejuni/coli* by the MPN enumeration method only, due to the expected high prevalence of *Campylobacter*.

Data Analysis:

Data was recorded as cfu/ml or MPN/ml, as appropriate for method. At the request of scientists who reviewed the study design, these counts were then converted to cfu/cm^2 or MPN/cm^2 (as appropriate) utilizing a broiler carcass weight to total surface area conversion formula reported by N. L. Thomas[8], as follows:

$$\text{Total Surface Area } (cm^2) = 0.87w + 635$$

where w is the eviscerated carcass weight expressed in grams. For example, for 10 cfu/ml recovered in the 400 ml rinse fluid from a 1500 g broiler carcass, the per cm^2 result would be calculated as follows:

$$\frac{\text{Total cfu}}{\text{Total Surface Area}} = \frac{\text{\# cfu/ml recovered x ml used to rinse}}{(0.87 \text{ x carcass weight in grams}) + 635} = \frac{10 \text{ cfu/ml x } 400 \text{ ml}}{(0.87 \text{ x} 1500\text{g}) + 635} = 2.1 \text{ cfu/cm}^2$$

The volume of fluid used to rinse the carcass, 400 ml (as specified by the protocol), was used in the calculations. Each sample result was calculated individually and then the overall mean results per cm^2 were determined.

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 in the broiler carcass rinse fluids. Tables 2/2a and Figure 2 present the mean level of selected microorganisms recovered from the broiler carcasses that tested positive for the particular microorganism. The mean levels in Tables 2/2a 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 1912 cfu per ml. Tables 3/3a - 7/7a and Figures 3 - 7 show the frequency within which all samples enumerated for each microorganism or group of microorganisms fall within specified intervals. Tables 8/8a - 10/10a and Figures 8 - 10 show the frequency within which only the positive samples enumerated for each microorganism fall within the specified intervals. Following is a brief summary of the results.

Viable aerobic bacteria (Aerobic Plate Count @ 35°C) were recovered from 100.0% of the 1,297 broiler carcasses analyzed in this program (Table 1, Figure 1). Coliforms were recovered from 99.9% and *E. coli* (Biotype I) was recovered from 99.6% of the 1,297 broiler carcasses. *C. perfringens* was recovered from 42.9% of the 1,297 broiler

carcasses; *S. aureus* was recovered from 64.0%; *L. monocytogenes* was recovered from 15.0%; *C. jejuni/coli* was recovered from 88.2%; and *Salmonella* was recovered from 20.0%. *E. coli* O157:H7 was not recovered from any of the 1,297 broiler carcasses analyzed.

In broiler carcass rinse fluids that tested positive, the geometric mean of the Aerobic Plate Count @ 35°C was 1912 cfu/ml (Table 2, Figure 2); the geometric mean of coliforms was 60 cfu/ml; and the geometric mean of *E. coli* (Biotype I) was 32 cfu/ml. When positive for a specific pathogen, the geometric mean in the carcass rinse fluids was: 7.2 cfu/ml *C. perfringens*; 13 cfu/ml *S. aureus*; 0.13 MPN/ml *L. monocytogenes*; 21 MPN/ml *C. jejuni/coli*; and 0.16 MPN/ml *Salmonella*.

When the carcass rinse fluids were tested for indicator organisms (Tables 3-5, Figures 3-5), 99.5% had aerobic plate counts (APC @ 35°C) of 100,000 or less colony forming units (cfu) per ml; 97.4% contained 1,000 or fewer coliforms per ml; and 98.0% contained 1,000 or fewer *E. coli* (Biotype I) per ml. 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: 870 cfu/ml for *C. perfringens*; 3,600 cfu/ml for *S. aureus*; 241 MPN/ml for *L. monocytogenes*; 230,000 MPN/ml for *C. jejuni/coli*; and 280 MPN/ml for *Salmonella*.

When the bacterial counts per ml carcass rinse fluid were converted to counts per square centimeter broiler carcass utilizing N. L. Thomas' formula[8], the geometric mean of the APC @ 35°C counts was 396 cfu/cm^2 (Table 2a). Of the samples that tested positive, the geometric mean of total coliforms was 12 cfu/cm^2; of *E. coli* (Biotype I), 6.7 cfu/cm^2; *C. perfringens*, 1.5 cfu/cm^2; *S. aureus*, 2.7 cfu/cm^2; *L. monocytogenes*, 0.025 MPN/cm^2; *C. jejuni/coli*, 4.4 MPN/cm^2; and *Salmonella*, 0.033 MPN/cm^2.

Tables 3a - 5a show the distribution of levels of indicator organisms on the broiler carcasses. Of all 1,297 carcasses tested, 99.9% had aerobic plate counts (APC @ 35°C) of 100,000 or less colony forming units per square centimeter (cfu/cm^2); 99.2% had 1,000 or fewer total coliforms/cm^2; and 99.4% had 1,000 or fewer *E. coli* Biotype I cfu/cm^2. Biotype I *E. coli* are generally considered to be non-pathogenic.

The highest level detected for each of the various pathogens was 284 cfu/cm^2 for *C. perfringens*; 676 cfu/cm^2 for *S. aureus*; 51 MPN/cm^2 for *L. monocytogenes*; 34,237 MPN/cm^2 for *C. jejuni/coli*; and 66 MPN/cm^2 for *Salmonella* (Tables 6a -10a).

None of the six pathogens, *C. perfringens*, *S. aureus*, *L. monocytogenes*, *C. jejuni/coli*, *E. coli* O157:H7 and *Salmonella*, were recovered from 28 (2.2%) of the 1,297 broiler carcasses tested (Table 11, Figure 11). Two hundred fifty-seven (19.8%) of the broiler carcasses contained only one pathogenic bacterial species, whereas 498 (38.4%)

contained two species, 343 (26.4%) contained three species, 152 (11.7%) contained four species and only 19 (1.5%) broiler carcasses contained a total of five species. No broiler carcasses tested contained all 6 pathogenic species.

DISCUSSION

This manuscript presents the primary goal of this program: a microbial profile of broiler chicken carcasses that includes the number and types of microorganisms recovered. Current procedures in use in federally inspected establishments are generally unable to completely remove viable bacteria from broiler carcasses during slaughter and evisceration operations. The microbial levels found in this study agree with the National Academy of Sciences report in 1985[4], which indicated that microorganisms are normally found on freshly processed carcasses at 10^3 to 10^4 cfu/cm^2, and include a variety of species. The presence of pathogenic bacteria on broiler chickens emphasizes the need for proper refrigeration, handling and cooking of chicken products throughout the food chain. However, the levels of pathogenic bacteria found were such that recommended cooking temperatures would render product produced from these broiler chickens safe to consume.