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Nationwide Young Turkey Microbiological Baseline Data Collection Program

August 1996 - July 1997

FOREWORD

This publication is a compilation of data obtained from the Nationwide Young Turkey Microbiological Baseline Data Collection Program for the period August 1996 through July 1997. The program was initiated to estimate the prevalence and levels of bacteria of public health concern on young turkey 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 YOUNG TURKEY MICROBIOLOGICAL BASELINE
DATA COLLECTION PROGRAM
August 1996 - July 1997**

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**NATIONWIDE YOUNG TURKEY MICROBIOLOGICAL
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EXECUTIVE SUMMARY

From August 1996 through July 1997, 1,221 young turkey 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 turkey carcasses as currently produced. The establishments in the program are responsible for approximately 99% of all turkeys slaughtered in the U.S. under Federal inspection. The turkey 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 29.2% of the 1,221 turkey carcasses analyzed, *Staphylococcus aureus* was recovered from 66.7%, *Listeria monocytogenes* was recovered from 5.9%, *Campylobacter jejuni/coli* was recovered from 90.3%, and *Salmonella* was recovered from 18.6%. *Escherichia coli* O157:H7 was not recovered from any of the 1,221 turkey 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 99.4% of the samples, 95.6% contained 1,000 or fewer total coliform cfu/ml, and 97.4% contained 1,000 or fewer *Escherichia coli* (Biotype I) cfu/ml. When converted to cfu per square centimeter turkey carcass surface area (cfu/cm²), aerobic plate counts of 100,000 or fewer cfu/cm² were found on 99.8% of the turkey carcasses, 99.4% contained 1,000 or fewer cfu/cm² total coliforms, and 99.6% contained 1,000 or fewer *E. coli* (Biotype I) cfu/cm². 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 young turkeys can contain a variety of bacteria, including some pathogens. Refrigerated raw poultry will eventually undergo microbial spoilage even if 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 young turkeys 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 Young Turkey Microbiological Baseline Data Collection Program focused on establishing a microbiological baseline for turkey production. These results on the presence and quantity of selected microorganisms are expressed as a national average. Young turkeys were chosen as the target population because they constitute approximately 99 percent of all turkeys slaughtered. The data obtained provides a microbiological description or profile of turkeys 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⁾, broilers⁽³⁾ and market hogs⁽⁴⁾.

PROGRAM DESIGN

Establishments Included in the Sampling Frame:

There are approximately 80 turkey slaughter establishments currently under Federal inspection. These plants slaughtered more than 276 million young turkeys. Young turkey production accounted for nearly 99 per cent of all turkey production within federally inspected plants. Old turkeys and fryer-roaster turkeys accounted for the remaining 1 percent of production. Only young turkey production was considered in design of the study. Of these, only those establishments that slaughter more than 200,000 turkeys per year were included in the sampling frame. In this category were approximately 50 establishments, which accounted for more than 99.9 percent of all turkeys slaughtered. The remaining establishments were not included in the frame because many were very small and slaughtered young turkeys 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 1844 turkey carcasses was requested during the 52-week time frame of the study (approximately 35 per week). Of these, laboratory results were obtained for 1,221 turkey 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 young turkeys 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 turkey carcasses. The data obtained provide an indication of which microorganisms might be present on federally inspected turkey 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 young turkeys 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, or, in plants where young turkeys were hot boned, at the last readily accessible point prior to packing/cut-up.

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, "Young Turkey Sample Collection Procedures" (4/10/96). A sample consisted of one randomly selected whole turkey 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⁽⁵⁾. 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) (7)} 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 turkey carcass in 600 ml of sterile Butterfield's Phosphate Diluent⁽⁸⁾. The subsequent laboratory methods used to analyze the carcass rinse fluid were identical to those used in all prior 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 organism 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/cm² or MPN/cm² (as appropriate) utilizing turkey carcass weight to total surface area conversion formulas reported by N. L. Thomas⁽⁹⁾, as follows:

$$\text{For turkeys weighing less than 7 kg - Total Surface Area (cm}^2\text{)} = 0.45w + 1293$$

where w is the eviscerated carcass weight expressed in grams. For example, for 10 cfu/ml recovered in the 600 ml rinse fluid from a 1500 g turkey carcass, the per cm² result would be calculated as follows:

$$\frac{\text{Total cfu}}{\text{Total Surface Area}} = \frac{\# \text{ cfu/ml recovered} \times \text{ml used to rinse}}{(0.45 \times \text{carcass weight in grams}) + 1293} = \frac{10 \text{ cfu/ml} \times 600 \text{ ml}}{(0.45 \times 1500\text{g}) + 1293} = 3.0 \text{ cfu/cm}^2$$

$$\text{For turkeys weighing more than 7 kg - Total Surface Area (cm}^2\text{)} = 0.13w + 3480$$

where w is the eviscerated carcass weight expressed in grams. For example, for 10 cfu/ml recovered in the 600 ml rinse fluid from a 8500 g turkey carcass, the per cm² result would be calculated as follows:

$$\frac{\text{Total cfu}}{\text{Total Surface Area}} = \frac{\# \text{ cfu/ml recovered} \times \text{ml used to rinse}}{(0.13 \times \text{carcass weight in grams}) + 3480} = \frac{10 \text{ cfu/ml} \times 600 \text{ ml}}{(0.13 \times 8500\text{g}) + 3480} = 1.3 \text{ cfu/cm}^2$$

The volume of fluid used to rinse the carcass, 600 ml (as specified by the protocol), was used in the calculations. Each sample result was calculated individually and then the overall mean results per cm² 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 turkey carcass rinse fluids. Tables 2/2a and Figure 2 present the mean level of selected microorganisms recovered from the young turkey 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 2,090 colony forming units per ml (cfu/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 1,221 young turkey carcasses analyzed in this program (Table 1, Figure 1). Coliforms were recovered from 99.8% and *E. coli* (Biotype I) was recovered from 98.9% of the 1,221 young turkey carcasses. *C. perfringens* was recovered from 29.2% of the 1,221 young turkey carcasses; *S. aureus* was recovered from 66.7%; *L. monocytogenes* was recovered from 5.9%; *C. jejuni/coli* was recovered from 90.3%; and *Salmonella* was recovered from 18.6%. *E. coli* O157:H7 was not recovered from any of the 1,221 turkey carcasses analyzed.

In turkey carcass rinse fluids that tested positive, the geometric mean of the Aerobic Plate Count @ 35°C was 2,090 cfu/ml (Table 2, Figure 2); the geometric mean of coliforms was 49 cfu/ml; and the geometric mean of *E. coli* (Biotype I) was 26 cfu/ml. When positive for a specific pathogen, the geometric mean in the carcass rinse fluids was: 4.0 cfu/ml *C. perfringens*; 22 cfu/ml *S. aureus*; 0.14 MPN/ml *L. monocytogenes*; 1.2 MPN/ml *C. jejuni/coli*; and 0.11 MPN/ml *Salmonella*.

When the carcass rinse fluids were tested for indicator organisms (Tables 3-5, Figures 3-5), 99.4% had aerobic plate counts (APC @ 35°C) of 100,000 or less cfu/ml; 95.6% contained 1,000 or fewer coliforms per ml; and 97.4% 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: 3,250 cfu/ml for *C. perfringens*; 20,100 cfu/ml for *S. aureus*; 2.3 MPN/ml for *L. monocytogenes*; 4,600 MPN/ml for *C. jejuni/coli*; and 23 MPN/ml for *Salmonella*.

When the bacterial counts per ml carcass rinse fluid were converted to counts per square centimeter turkey carcass utilizing N. L. Thomas' formula⁽⁹⁾, the geometric mean of the APC

@ 35°C counts was 297 cfu/cm² (Table 2a). Of the samples that tested positive, the geometric mean of total coliforms was 7.0 cfu/cm²; of *E. coli* (Biotype I), 3.7 cfu/cm²; *C. perfringens*, 0.54 cfu/cm²; *S. aureus*, 3.2 cfu/cm²; *L. monocytogenes*, 0.02 MPN/cm²; *C. jejuni/coli*, 0.18 MPN/cm²; and *Salmonella*, 0.02 MPN/cm².

Tables 3a - 5a show the distribution of levels of indicator organisms on the turkey carcasses. Of all 1,221 carcasses tested, 99.8% had aerobic plate counts (APC @ 35°C) of 100,000 or less colony forming units per square centimeter (cfu/cm²); 99.4% had 1,000 or fewer total coliforms/cm²; and 99.6% had 1,000 or fewer *E. coli* Biotype I cfu/cm². Biotype I *E. coli* are generally considered to be non-pathogenic.

The highest level detected for each of the various pathogens was 383 cfu/cm² for *C. perfringens*; 3,703 cfu/cm² for *S. aureus*; 0.3006 MPN/cm² for *L. monocytogenes*; 981 MPN/cm² for *C. jejuni/coli*; and 4.9 MPN/cm² 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 32 (2.6%) of the 1,221 turkey carcasses tested (Table 11, Figure 11). Two hundred sixty-one (21.4%) of the turkey carcasses contained only one pathogenic bacterial species, whereas 557 (45.6%) contained two species, 292 (23.9%) contained three species, 74 (6.1%) contained four species and only 5 (0.4%) turkey carcasses contained a total of five species. No turkey carcasses tested contained all 6 pathogenic species.

DISCUSSION

This manuscript presents the primary goal of this program: a microbial profile of young turkey 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 turkey carcasses during slaughter and evisceration operations. The microbial levels found in this study agree with the National Academy of Sciences report in 1985⁽⁵⁾, which indicated that microorganisms are normally found on freshly processed carcasses at 10³ to 10⁴ cfu/cm², and include a variety of species. The presence of pathogenic bacteria on young turkeys emphasizes the need for proper refrigeration, handling and cooking of turkey products throughout the food chain. However, the levels of pathogenic bacteria found were such that recommended cooking temperatures would render product produced from these young turkeys safe to consume.

TABLES

Table 1. Prevalence of Selected Microorganisms in Turkey Carcass Rinse Fluids

Microorganism	Samples Analyzed	Samples Positive		SE ¹
		Number Positive	Percent Positive	
INDICATOR ORGANISMS				
Aerobic Plate Count @ 35°C	1221	1221	100.00	NA ²
Total Coliforms	1221	1218	99.8	0.1
<i>Escherichia coli</i> (Biotype I)	1221	1208	98.9	0.3
PATHOGENIC ORGANISMS				
<i>Clostridium perfringens</i>	1221	357	29.2	1.3
<i>Staphylococcus aureus</i>	1221	814	66.7	1.3
<i>Listeria monocytogenes</i>	1221	72	5.9	0.7
<i>Campylobacter jejuni/coli</i>	1221	1102	90.3	0.8
<i>Escherichia coli</i> O157:H7	1221	0	NA ²	NA ²
<i>Salmonella</i>	1221	227	18.6	1.1

¹ Standard Error using the binomial distribution.

² NA= Not applicable

Source: Nationwide Young Turkey Microbiological Baseline Data Collection Program (August 1996 - July 1997).