Enumeration of *Escherichia coli* Cells on Chicken Carcasses as a Potential Measure of Microbial Process Control in a Random Selection of Slaughter Establishments in the United States[▽]

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To evaluate whether the number of *Escherichia coli* bacteria in carcass rinses from chicken slaughter establishments could be monitored for the purpose of microbial process control, we drew a random sample from 20 of 127 large USDA-inspected operations. In 2005, every 3 months, two sets of 10 carcass rinses, 100 ml each, were collected from establishments, netting 80 sample sets from the rehang and postchill stages. *E. coli* and *Campylobacter* numbers and *Salmonella* prevalence were measured. Mixed-effect models were used to estimate variance of mean $\log_{10}$ *E. coli* cell numbers of 10-carcass rinse sample sets. Relationships between *E. coli* and *Campylobacter* and *Salmonella* were examined. For 10-carcass rinse sets, at both the rehang and postchill stages the mean $\log_{10}$ *E. coli* CFU/ml fit the logistic distribution better than the normal distribution. The rehang overall mean $\log_{10}$ *E. coli* was 3.3 CFU/ml, with a within-sample set standard deviation of 0.6 CFU/ml. The overall postchill mean $\log_{10}$ *E. coli* was 0.8 CFU/ml, with 13 establishments having mean $\log_{10}$ *E. coli* CFU/ml values of less than 1.0 and 7 having mean values of 1.2 or more. At the midpoint separating these establishments, a mean $\log_{10}$ *E. coli* CFU/ml of 1.1, the within-sample set standard deviation was 0.5 CFU/ml, with smaller standard deviations as means increased. Postchill sample sets with mean $\log_{10}$ *E. coli* counts less than or equal to 1.1 CFU/ml had lower overall prevalence of *Salmonella* and mean $\log_{10}$ *Campylobacter* CFU/ml than sample sets with higher means. These findings regarding reductions in *E. coli* numbers provide insight relevant to microbial process control.

Regulatory food microbiology standards are defined and enforced with the intent of protecting public health and maintaining consumer confidence in the safety of the food supply. Resource demands (22) and legal constraints (21) have hindered the U.S. Department of Agriculture (USDA) from enforcing its current *Salmonella* performance standard (3). For this reason, in 2004 the USDA requested guidance from its national scientific advisory committee on the possible use of *E. coli* numbers to monitor sanitary conditions during poultry slaughter (12). The committee acknowledged that, if valid, such a performance standard could facilitate inspection of slaughter processing establishments. The committee recommended studies to define how *E. coli* numbers vary in poultry carcass rinses during poultry processing by processing stage, time of year, and geographic region and with respect to foodborne pathogens.

The widespread presence and high numbers of generic *E. coli* bacteria on poultry entering the slaughter establishment (2, 5, 14) are suitable characteristics for an indicator organism used to monitor microbial control processes. The ease and lower cost (5, 13) of *E. coli* enumeration also allow more

observations than can be made when comparable resources are allocated for *Campylobacter* or *Salmonella* testing (15).

Regulatory agencies and food manufacturers have recognized the potential utility of *E. coli* numbers as a measure of slaughter process control. For example, USDA's hazard analysis and critical control point rule (3) specifies two criteria for evaluating process control: establishments are to maintain less than 100 CFU of *E. coli* per ml in 80% of poultry carcass rinses and never exceed 1,000 CFU/ml. Surveys have been performed to define precise *E. coli* performance criteria for poultry (5), to monitor microbial reduction during slaughter processing (6), and to validate interventions to reduce microbial numbers on poultry (20).

If generic *E. coli* numbers on poultry carcasses fit a parametric distribution, with a predictable mean and standard deviation, then carcasses could be monitored using a statistical process control plan. For example, if *E. coli* numbers decrease by an acceptable amount during processing to a reasonable level, then the process could be considered to be under control. Or a plan could be designed to monitor for acceptable occurrences of small, medium, and large deviations above a target *E. coli* number (7). If relationships were found between *E. coli* and *Campylobacter* numbers during chicken slaughter as well as *Salmonella* prevalence, they would further support the use of *E. coli* numbers as a measure of process control.

This study of a random sample of 20 large chicken slaughter operations located throughout the United States mea-

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sured microbial numbers at two processing line locations. Once a quarter, 10 carcass rinse samples were collected from both the post-feather-pick (rehang) and postchill locations. Rinses were examined to estimate mean *Salmonella* prevalence and *E. coli* and *Campylobacter* numbers by location within establishments. The primary objective was to assess whether the reduction in *E. coli* numbers between the rehang and postchill stages or numbers at the postchill location might have utility as a measure of process control during chicken slaughter. A related objective was to estimate values of parameters that could be used to design statistical process control plans (7).

MATERIALS AND METHODS

Sampling. All 127 large (i.e., 500 or more employees) USDA federally inspected young chicken slaughter establishments in operation in the autumn of 2004 were eligible for the study. Establishments were stratified by Food Safety and Inspection Service (FSIS) region, and a random sample of about one in six establishments was drawn in each region to yield a sample of 20 establishments. The random sample was drawn using the SAS Procedure Proc Surveyselect (SAS, version 9.1; SAS Institute, Cary NC), without replacement. Selected establishments were located in 13 states (Alabama, Arkansas, California, Delaware, Georgia, Indiana, Missouri, North Carolina, South Carolina, Tennessee, Texas, Virginia, and West Virginia) and represented eight integrated broiler companies. The survey provided a nationally distributed sample of typical chicken slaughter processes in large USDA-inspected operations during fiscal year 2005.

In each establishment, once every 3 months, FSIS personnel collected a set of 10 carcasses at the rehang (postpick) and postchill stages from one flock. Carcasses were collected using commercial kits (Solar Biologicals, Ogdensburg, NY). Each carcass was placed in a specially manufactured plastic bag, and 100 ml of sterile buffered peptone water was added. The collector shook the bag by hand for 1 min and removed the carcass aseptically. The full 100 ml of rinse was typically recovered with little or no excess rinse following the procedure. Vials were packaged in an insulated shipping container lined with six previously frozen 500-ml cold packs and shipped to the Agricultural Research Service (ARS) Bacterial Epidemiology and Antimicrobial Resistance Laboratory in Athens, GA, by overnight courier. Rinse temperature was measured upon receipt at the laboratory. All rinses used in the study arrived at the laboratory at temperatures between 0º and 9°C. In the first weeks of the study, however, rinses from five establishments were discarded because they were received at or above 10°C. In these establishments, rinses were collected 1 year later to provide data for four quarters. Two postchill rinses were lost, yielding 798 postchill carcass rinses.

Microbiology. (i) Generic *E. coli*. *E. coli* bacteria were enumerated by inoculating a 10-fold serial dilution of rinses onto *E. coli* Petrifilms (3 M Corporation, St. Paul, MN). Sterile saline (0.85%) was used for dilution in accordance with manufacturer's instructions. After incubation at 35°C for 24 h, typical *E. coli* colonies were counted.

(ii) *Campylobacter*. Numbers of CFU (CFU/ml) of *Campylobacter* bacteria were estimated by directly plating serial dilutions of carcass rinse on Campy Cefex agar plates (19). In the second, third, and fourth quarters of the study, in order to improve sensitivity of detection for low numbers of *Campylobacter* bacteria at the postchill stage, four 0.25-ml aliquots of undiluted rinse were plated onto four agar plates. Plates were incubated at 42°C for 48 h under the following atmospheric conditions: 5% O_2, 10% CO_2, and 85% N_2. Presumptive *Campylobacter* colonies were confirmed based on cellular morphology and motility under phase-contrast microscopy, followed by latex bead antigen agglutination testing of thermophillic *Campylobacter* (Microgen Bioproducts Ltd., Camberley, Surrey, United Kingdom).

(iii) *Salmonella*. Culture for *Salmonella* was conducted using standard FSIS methods for isolation from poultry rinses (22). A 30-ml aliquot of each carcass rinse was added to sterile buffered peptone water and incubated at 35 ± 2°C for 20 to 24 h. After enrichment, gene amplification (BAX; E. I. du Pont de Nemours and Company, Wilmington, DE) was conducted on lysed cells. The PCR-positive rinses were plated for isolation, as follows: 0.5 ml of preenriched broth was transferred to 10 ml of tetrathionate broth (Becton Dickinson, Sparks, MD), and 0.1 ml was transferred to 10 ml of Rappaport-Vassiliadis R10 broth (Becton Dickinson) and incubated aerobically at 37°C for 24 h. Next, 1 loopful each of tetrathionate broth and Rappaport-Vassiliadis broth was streaked on

modified lysine iron agar (Oxoid, Basingstoke, United Kingdom) and brilliant green sulfa agar (Becton Dickinson) and incubated aerobically at 37°C for 24 h. After incubation, a well-isolated colony with typical *Salmonella* sp. morphology was picked from the modified lysine iron agar and brilliant green sulfa agar and screened on triple sugar iron agar (Becton Dickinson) and lysine iron agar (Becton Dickinson) slants. Isolates with typical *Salmonella* biochemical reactions were confirmed to be *Salmonella* based on detection of somatic (Becton Dickinson, Sparks, MD) and flagellar (latex agglutination; Microgen, Camberley, United Kingdom) antigens.

Statistics. To estimate the number of *Campylobacter* and *E. coli* CFU per ml of rinse, duplicate plates were inoculated for each serial dilution of the carcass rinse. The $\log_{10}$ CFU/ml values of both *Campylobacter* and *E. coli* were estimated based on means from duplicate plates. Because the logarithm of zero is undefined, when no cells were present on either zero dilution plate of the rinse sample, the results for *Campylobacter* or *E. coli* were set to 0.25 CFU/ml (i.e., one half of the limit of detection, or 0.5 CFU/ml).

Distribution of $\log_{10}$ *E. coli* numbers. The mean $\log_{10}$ CFU/ml of *E. coli* was determined for each 10-carcass rinse set collected in one establishment at one processing location on 1 day. For both the rehang and postchill rinses, the standardized mean $\log_{10}$ CFU/ml values of *E. coli* for 10-carcass rinse sample sets were calculated for comparison with fitted logistic and normal distributions. The overall mean was subtracted from the sample set mean, and the difference was divided by the predicted standard deviation, adjusted for finite population size. The Kolmogorov-Smirnov test was used to assess goodness of fit of data to normal and logistic distributions (18).

To estimate within-set standard deviations of the 10-carcass sample sets for $\log_{10}$ *E. coli* CFU/ml values, six rehang sample results were excluded as outliers, for a final data set of $\log_{10}$ *E. coli* CFU/ml values at the rehang stage consisting of 794 of 800 observations. To estimate within-set standard deviations of the 10-carcass sample sets at postchill stage, the analysis was restricted to 66 of 80 postchill 10-carcass rinse sample sets in which the mean $\log_{10}$ CFU/ml for *E. coli* exceeded 0.5, i.e., *E. coli* was detected in the majority of carcass rinses. Two rinses were lost during shipping, and two others were excluded as outliers. Thus, the standard deviation and distribution of postchill *E. coli* numbers were based on observations for 656 rinses.

Estimate of variances of $\log_{10}$ CFU/ml *E. coli* numbers within the 10-carcass sample sets at the rehang and postchill stages were obtained using maximum-likelihood estimation (8).

Mixed-effect models estimated standard deviations by the mean $\log_{10}$ *E. coli* CFU/ml of 10-carcass rinse sample sets. The models accounted for nested structure of data: individual rinses, 10-carcass sets, and establishments (11), as reported on USDA's website (23). Linear regression was used to evaluate the standard deviation of $\log_{10}$ *E. coli* CFU/ml with increasing 10-carcass rinse set means. Regression models compared reductions in mean $\log_{10}$ CFU/ml for *Campylobacter* and *E. coli* in flock-matched rehang and postchill sets (4). Analyses were performed with SAS, version 9.1 (SAS, Cary, NC), S-PLUS (Insightful Corporation, Seattle, WA), and WinBugs, version 1.4 (MRC Biostatistics Unit, Cambridge, United Kingdom).

RESULTS

***E. coli* distributions at the rehang and postchill locations.** Overall, 796 of 800 (99.5%) rehang rinses and 691 of 798 (87%) postchill rinses tested positive for *E. coli*. Standardized $\log_{10}$ *E. coli* CFU/ml values of both rehang and postchill 10-carcass rinse sample sets fit the logistic distribution better than the normal distribution due to kurtosis in the observed distributions (Fig. 1). The *P* values for the Kolmogorov-Smirnov test of goodness of fit to the logistic and normal distributions at the rehang stage were 0.017 and 0.003, respectively, and at postchill stage they were 0.059 and 0.015, respectively.

For rehang rinses, the overall mean $\log_{10}$ CFU/ml for *E. coli* was 3.3 CFU/ml (Table 1). The within-set standard deviation of the 10-carcass sample set was approximately 0.6 $\log_{10}$ CFU/ml. Among rehang 10-carcass rinse sample sets, the standard deviations of $\log_{10}$ *E. coli* CFU/ml remained stable as the mean $\log_{10}$ *E. coli* CFU/ml increased (data not shown).

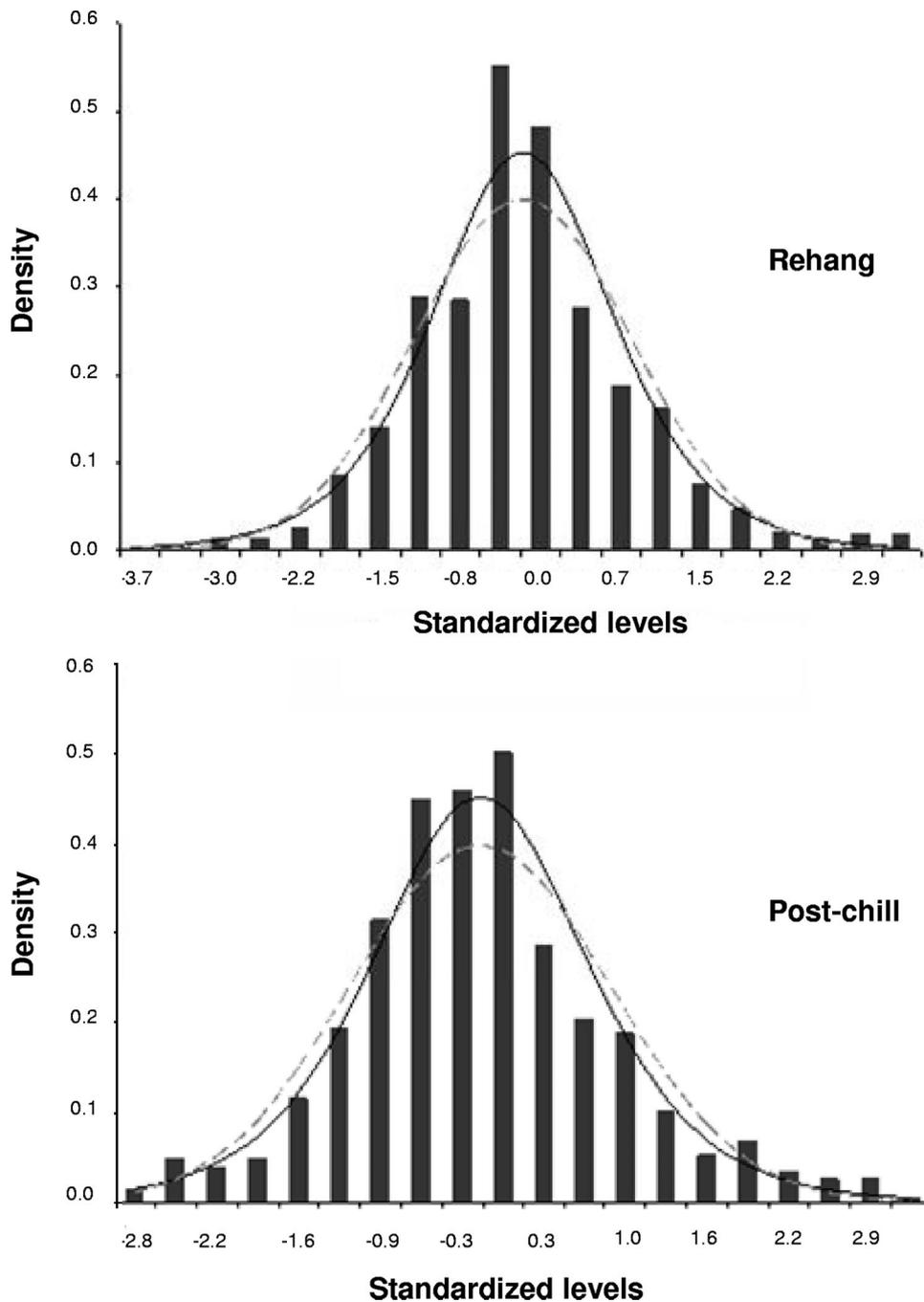For postchill rinses the overall mean $\log_{10}$ *E. coli* CFU/ml

FIG. 1. Standardized mean $\log_{10}$ *E. coli* CFU/ml values of 10-carcass rinse sets collected at rehang and postchill stages with fitted distributions Solid lines, logistic distribution; dotted line, normal distribution.

was 0.8 CFU/ml. Among postchill rinses the standard deviations of $\log_{10}$ *E. coli* CFU/ml values slightly decreased with increases in the mean $\log_{10}$ *E. coli* CFU/ml (data not shown). The within-set standard deviation of the 10-carcass sample sets for *E. coli* at the postchill stage was approximately 0.5 $\log_{10}$ CFU/ml when the 10-carcass rinse sample set mean was 1.1 $\log_{10}$ CFU/ml, a value of potential relevance for process control (below).

Relationships between *E. coli* and other bacteria. The overall prevalence of *Salmonella* at the rehang stage was 71%, and

the mean for *Campylobacter* was 2.5 $\log_{10}$ CFU/ml. The overall *Salmonella* prevalence at the postchill stage was 21%, and the mean $\log_{10}$ CFU/ml for *Campylobacter* was 0.02 $\log_{10}$ CFU/ml. In each quarter, the mean $\log_{10}$ CFU/ml for *E. coli* was numerically higher than that for *Campylobacter* at both the rehang and postchill stages.

At postchill, 13 establishments had overall mean *E. coli* numbers below 1.0 $\log_{10}$ CFU/ml, and 7 had mean numbers of 1.2 $\log_{10}$ CFU/ml or more (data not shown). Thus, these establishments fell into two groups separated by a midpoint

TABLE 1. Mean *E. coli* and *Campylobacter* counts as well as *Salmonella* prevalence for rehang and postchill chicken carcass rinses by quarter of collection and for the year

Study quarter	Rehang rinse data				Postchill rinse data			
	No. of rinses	*E. coli* count ($\log_{10}$ CFU/ml)	*Salmonella* prevalence (%)	*Campylobacter* count ($\log_{10}$ CFU/ml)	No. of rinses	*E. coli* count ($\log_{10}$ CFU/ml)	*Salmonella* prevalence (%)	*Campylobacter* count ($\log_{10}$ CFU/ml)
1	200	3.3	72	2.7	200	0.7	29	−0.05
2	200	3.2	76	2.3	199	1.0	20	0.01
3	200	3.3	70	2.7	200	0.9	17	0.35
4	200	3.3	74	2.3	199	0.7	18	−0.23
Overall[a]	800	3.3	71	2.5	798	0.8	21	0.02

[a] October 2004 to October 2005.

postchill mean value of 1.1 $\log_{10}$ *E. coli* CFU/ml. Compared to the seven establishments with postchill mean $\log_{10}$ *E. coli* CFU/ml values greater than 1.1, a larger proportion of the 13 establishments with lower means had postchill mean $\log_{10}$ *Campylobacter* CFU/ml values less than 0.0 and postchill *Salmonella* prevalences less than 20% (Table 2). However, these associations, based on a sample size of 20, were not statistically significant at a *P* value of <0.05.

Similarly, although not statistically significant, in postchill sample sets, *Salmonella* prevalence and *Campylobacter* numbers were sometimes higher when mean $\log_{10}$ *E. coli* CFU/ml exceeded 1.1. In 32 sample sets with higher *E. coli* numbers, the mean $\log_{10}$ *Campylobacter* CFU/ml was 0.26 compared to 0.14 in 48 sample sets with lower *E. coli* numbers. *Salmonella* prevalence was 27% compared to 17% in the same respective sample sets.

Reduction of *E. coli* and *Campylobacter* numbers. Both mean $\log_{10}$ CFU/ml values for *Campylobacter* and *E. coli* decreased between the rehang and postchill stages (Table 1). In a total of 11 matched 10-carcass sample sets collected from the rehang and postchill stages in one operation on the same day, *Campylobacter* was detected in each rinse. In 10 of these 11 matched sample sets, the mean reduction in *Campylobacter* numbers was greater than that of *E. coli* numbers (Fig. 2).

DISCUSSION

In this study of carcass rinses from 20 randomly selected chicken slaughter establishments, mean $\log_{10}$ *E. coli* CFU/ml values in the rehang and postchill rinse sample sets fit parametric distributions. In addition, mean $\log_{10}$ *E. coli* numbers at the postchill stage were consistent with those reported in other surveys performed around the time of this study (2, 17). A postchill mean $\log_{10}$ *E. coli* CFU/ml value of 1.1 provided a useful reference for the design of a process control plan (7), defining two distinct groups of establishments, those with higher versus lower means. This value also suggested a possible tolerance above the mean for the purpose of process control. With additional information confirming expected *E. coli* numbers during poultry processing, control plans may be developed that define acceptable frequencies of small, medium, and large deviations above the process mean (7) and other quality control measures (e.g., moving averages or the CUSUM method).

A statistical process control plan for poultry slaughter sanitation based on *E. coli* numbers of carcass rinses at the postchill stage might have practical merits for several reasons. The high yield (5) of *E. coli* enumeration compared to labor- and resource-intensive pathogen testing protocols (15) is advantageous for the purpose of statistical process control (7). For example, fewer than half of the postchill

TABLE 2. Overall mean $\log_{10}$ *Campylobacter* CFU/ml and *Salmonella* prevalence in chicken carcass rinses at the postchill stage stratified by plant mean $\log_{10}$ *E. coli* counts

Parameter	No. (%) of plants by the indicated mean $\log_{10}$ *E. coli* CFU/ml		Total no. of plants (%) positive for *E. coli*
	≤1.1	>1.1	
Overall *Campylobacter* count			
$\log_{10}$ CFU/ml ≤0.0	8 (62)	3 (43)	11 (55)
$\log_{10}$ CFU/ml >0.0	5 (38)	4 (57)	9 (45)
Overall *Salmonella* prevalence			
<20%	7 (54)	3 (43)	10 (50)
>20%	6 (46)	4 (57)	10 (50)

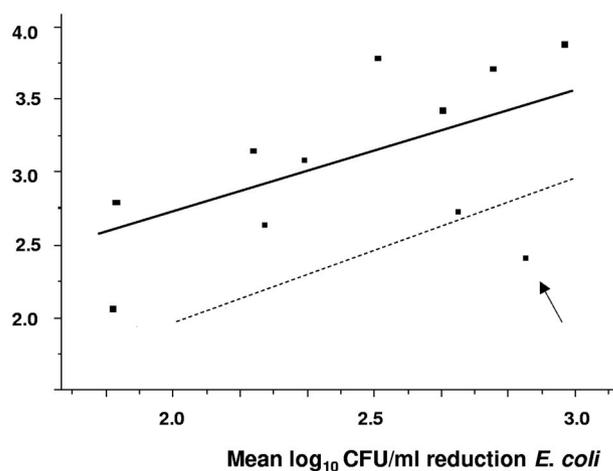**Mean $\log_{10}$ CFU/ml reduction *Campylobacter***

FIG. 2. Reduction of mean $\log_{10}$ *Campylobacter* CFU/ml versus mean $\log_{10}$ *E. coli* CFU/ml values from the rehang to postchill stage for 11 matched 10-rinse sample sets in which *Campylobacter* was detected in all rinses. The arrow denotes one instance with a greater reduction in the $\log_{10}$ *E. coli* value than the value for *Campylobacter*. Solid line, fitted linear regression line for data; dotted line, 45° line.

rinses described in the present report tested positive for either *Salmonella* or *Campylobacter*. In contrast, *E. coli* was detected in almost 90% of these rinses. Some variability of *E. coli* numbers was, however, noted across study quarters. More studies are therefore recommended of environmental factors that could affect *E. coli* numbers in poultry rinses.

The evidence regarding relationships between indicator organisms and pathogens on poultry carcasses is equivocal. Although *E. coli*, *Salmonella*, and *Campylobacter* are often present in the chicken gastrointestinal tract (10) and on carcasses, (5) if there are relationships between their presence on carcasses, they may be altered by environmental factors. These factors include the pathogen loads on chickens, which are known to vary by flock (9). The effectiveness of pathogen controls such as feed withdrawal prior to slaughter can also be influenced by host factors, such as the age of the bird (13). As another example, disease conditions within flocks (16) are reported to alter microbial numbers at the postchill stage. In addition, some slaughter plant interventions, such as carcass chilling, can result in differential reductions in *Campylobacter* and *E. coli* numbers (14). Cautious interpretation is therefore advised regarding relationships between *E. coli* levels and food-borne pathogens in this study. Nonetheless, in both establishments and sample sets, overall *Salmonella* prevalence and mean *Campylobacter* numbers were frequently, but not uniformly, higher when the postchill mean $\log_{10}$ *E. coli* CFU/ml exceeded 1.1. To further elucidate relationships between *E. coli* and pathogens, additional surveys with larger sample size are therefore recommended.

We have previously reported that poultry processing significantly reduces *Campylobacter* counts in carcass rinses (1). In this report overall mean *Campylobacter* numbers at both the rehang and postchill stages were lower than those of *E. coli*. Even so, reductions in *Campylobacter* numbers between these processing locations were generally greater than those for *E. coli* when comparisons were possible. Thus, we suggest that, in most instances, processes that lower the *E. coli* level at postchill stage are unlikely to increase *Campylobacter* numbers. Practical reasons for monitoring reductions of *E. coli* over *Campylobacter* include the relative ease of enumeration and lower cost (2).

Limitations of this study included the number of establishments and the times that each establishment was sampled. In addition, human and laboratory resources precluded enumeration of *Salmonella*. A strength of this study was the relatively large number of carcass rinses within sample sets that permitted reasonable estimates of variance. In summary, findings of this study suggest that *E. coli* numbers in postchill chicken carcass rinses may have features of value for process control plans (7). Specifically, *E. coli* numbers were reduced during processing and fit the logistic distribution at the postchill stage, with stable means and defined standard deviations. The study was not designed to evaluate efficacy of processing interventions (20) or the effect of microbial loads in live birds (9). Rather, microbial numbers were measured at the rehang and postchill stages in a randomly selected group of U.S. chicken processing operations. Additional studies are recommended of *E. coli* numbers in poultry carcass rinses, environmental factors that could affect these numbers, and possible relationships with pathogens.

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