Improvements for Poultry Slaughter Inspection

Appendix H – Data Analyses Supporting Proposed Performance Standards

APPENDIX H – DATA ANALYSES SUPPORTING PROPOSED PERFORMANCE STANDARDS

Under current regulations, each official establishment that slaughters poultry must sample whole carcasses and test for generic *Escherichia* (*E.*) *coli* at the end of the chilling process or, if that is impractical, at the end of the slaughter line. Generic *E. coli* are enteric bacteria found in the intestines of animals. Although data indicate that generic *E. coli* is not a good indicator of *Salmonella*, the presence of generic *E. coli* at high levels indicates the presence of intestinal material, or filth, and could be a measure of sanitation. Measuring *E. coli* at the end of the chilling process or the end of the slaughter line could be a means to verify the efficiency of microbial process controls that are designed to ensure sanitary conditions on carcasses. The FSIS, therefore, is considering having all poultry slaughter establishments meet a new performance standard for generic *E. coli*, requiring establishments to measure generic *E. coli* at two points in the process: at re-hang and at post-chill. Those data could be used to verify that either acceptable levels of generic *E. coli* are achieved at re-hang and post-chill, or that an acceptable log reduction in generic *E. coli* is met. Distribution of measured generic *E. coli* levels at particular points in processing, rather than the overall distribution of results could be used as an indication of insanitary conditions. In addition, generic *E. coli*, although not an indicator of absolute incidence or levels of pathogens, could potentially be used as an indicator of reductions in pathogens.

The FSIS and the Agricultural Research Service (ARS) conducted a study of 20 establishments to measure generic *E. coli* distributions for the purpose of relating *E. coli* to sanitation, and to compare reductions in generic *E. coli*, *Salmonella*, and *Campylobacter* for the same flock from re-hang (post-pick) to post-chill. The results of the analyses are presented in this appendix.

Background

Generic *E. coli* is an enteric organism, and as such, it represents undesirable material indicative of insanitary conditions on carcasses. It is ubiquitous, making it a good measure of microbial process control if present at "too high" a level, which could be defined through the performance standard. Regardless of whether *Salmonella* or *Campylobacter* levels are low, high generic *E. coli* levels would indicate insanitary conditions and poor microbial process control.

In order to examine the levels of generic *E. coli* at different points in processing, and the relationships between reductions in generic *E. coli*, *Salmonella*, and *Campylobacter*, FSIS conducted a 20-establishment study with the ARS. A random sample of 20 large establishments (about 1 in 6) was selected. Every 3 months, FSIS personnel collected 10 broiler-carcass 100-ml rinse samples at both the re-hang (post-pick) and post-chill locations from the same flock, representing a "moment" of processing. For each location, there were 80 sets of ten 100-ml rinse samples. Further details of the ARS methods are presented in Attachment 1.

Defining the Target Cumulative Distribution Function F

In order to examine the data in the context of potentially setting performance standards, a parametric analysis of the full distribution (F), rather then just percentiles of a distribution, could

be used. By using the full distribution, the operating characteristics of compliance procedures are designed to reflect the nonpresumptive nature of the evaluation of the process. Specifically, for the analysis it was stipulated, that, based on FSIS sampling, there would be about a 95 percent, or slightly greater, probability that an establishment would not fail any of the sampling plan's rules if the "true" distribution of the (measured) *E. coli* levels were equal to F. It is also important to consider whether the measure used is robust. A robust measure is one in which the impacts on the measure of two results for which the difference is "small" are, for the most part, nearly the same; and the impacts on the measure of two results that differ by a large amount are, for the most part, quite different. Using a count of the number of observations above a certain value (or two values, such as m [= 2 log] and M [= 3 log] in the 3-attribute sampling plan of the present regulation), as was done previously for a generic *E. coli* standard, is not a robust measure. Through the use of the distribution of function F, FSIS could develop performance standards that are more robust than the present rules. The cumulative distribution function (CDF) F is defined in two stages. The first stage specifies the median of the distribution. The next stage defines the actual form of the distribution with the given median.

Post-Chill

To determine F for post-chill, first, the median of the distribution must be defined. To do that, the levels of *E. coli* per ml, were transformed by the logarithm base 10; for non-detect sample results, ½ the level of detection (LOD) was used. For each sample, two 1-ml plates were used, so that the LOD is equal to 0.5. Mean log values were computed for each sample set (10-carcass-rinse-sample-set). Thus, there were 80 mean values. For each sample set, the *Salmonella* and *Campylobacter* incidences were computed, as well as the mean log of the *Campylobacter* levels, using the same rule for ND values as above for *E. coli*.

Figure 1 shows a plot of sample set-specific logit (*Salmonella* incidence) versus means of $\log_{10}$ *E. coli* levels. For *Salmonella* incidence = 0 or 1, a logit value was assigned of -3 or 3, respectively. **Figure 2** shows a plot of sample set-specific means of the $\log_{10}$ of *Campylobacter* levels versus means of $\log_{10}$ *E. coli* levels. From the data it can be noted that the four highest 10-carcass-rinse-sample set-specific *Salmonella* incidence ($\geq$ 80 percent) had corresponding means of log of *E. coli* levels greater than 1.1 log (> 12.5 CFU/ml). The three highest 10-carcass-rinse-sample set-specific mean log *Campylobacter* levels had corresponding means of log of *E. coli* levels greater than 1.1 log. It was noted that of the 80 sets specific mean values, 32 were above 1.1 $\log_{10}$ and 48 were below (about 60 percent were less than 1.1). A gap in the distribution of the establishment-specific means of the log *E. coli* levels (averaged over the four sets for each establishment) was found between 1 and 1.2 (with seven establishment – specific means greater than 1.0). Therefore, 1.1 is set to be the median value for F.

The actual performance standard is stated in terms of a distribution, with CDF F. The shape or form of the distribution can be determined by examining the distribution of log *E. coli* levels within 10-carcass-rinse-sample-sets. The sample set-specific standard deviations decreases with increasing mean levels (see **Figure 3**). For post-chill, analysis of variance was performed deleting data from sets with mean $\log_{10}$ less than 0.03, and with standard deviation greater than 1.2 (Figure 3). This eliminates most of the data with ND results. Data from 62 sets remained. Two other data points whose results were about 2.3 $\log_{10}$ greater than corresponding set-specific mean values, excluding the outlier points, were deleted as outlier values. Thus, there were

616 data points used in the analysis (from the original 798 results), because 2 samples results were not reported.

The basic analysis of variance model was:

$$y_{jk} = \mu_j + \varepsilon_{jk},$$

where y_{jk} is the $\log_{10}$ of the *E. coli* result, for the k^{th} sample within the j^{th} samples set, μ_j is the expected value of y_{jk} within the j^{th} set, and ε_{jk} is a random error term, with mean = 0 and standard deviation

$$\sigma_j = be^{-c\mu_j}$$

for j = 1, …, 62. Estimates were derived using maximum likelihood estimation (MLE).

This basic model has 64 parameters. The estimates of b and c were: b = 6,536, and c = 0.3093. The predicted standard deviation at 1.1 is 0.4651. Treating the parameters, μ_j, as a random factor from a common distribution with mean equal to μ and standard deviation equal to σ_μ, reduces the number of parameters to 4. The estimates of b and c for this model were: b = 6,882, and c = 0.3077. The predicted standard deviation at 1.1 is 0.4906. A model treating the parameters μ_j as random factor taking establishment into account, that is,

$$\mu_j = \mu + \alpha_p + \beta_{pt},$$

where α_p is a random error term associated with the p^{th} establishment (between establishment error) with standard deviation σ_p, and β_{pt} is a random error term associated within the p^{th} establishment with standard deviation σ_{pt}, has 5 parameters. The estimates of b and c for this model were: b = 6,843, and c = 0.2963. The predicted standard deviation at 1.1 is 0.4940.

For F, the last model will be used so that the predicted standard deviation when the mean of the log *E. coli* measured values is equal to 1.1 is about 0.494. A standardized distribution of the log *E. coli* levels, derived by pooling over the results, dividing the difference between the individual log values, minus the mean value for the set, by the predicted standard deviation, was reasonably approximated by a logistic distribution (see **Figure 4**).

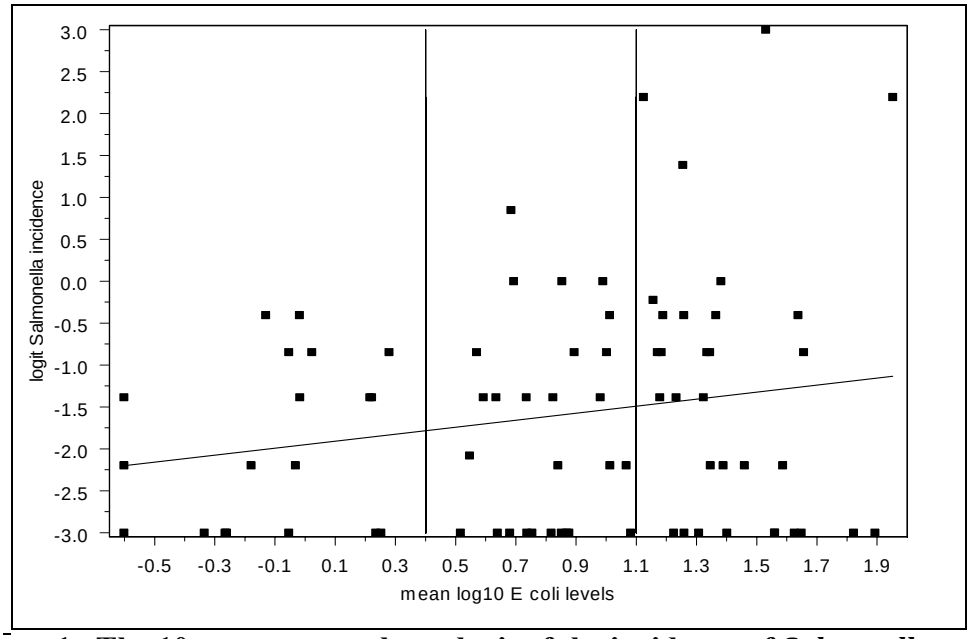

Figure 1. The 10-carcass sample set logit of the incidence of *Salmonella* versus the mean of the $\log_{10}$ *E. coli* levels at post-chill. There are 80 data points for the 20 establishments, 4 sets per establishments. For incidence of 0 or 1, a logit value of -3 or 3, respectively, was assigned (for graphical purposes only). The vertical lines are at 0.4 and 1.1 log, representing "perceived" gaps in the data. The OLS linear regression line (created by the S-Plus program) is shown based on the 80 data points, and not taking establishment, season, or treatment into account.

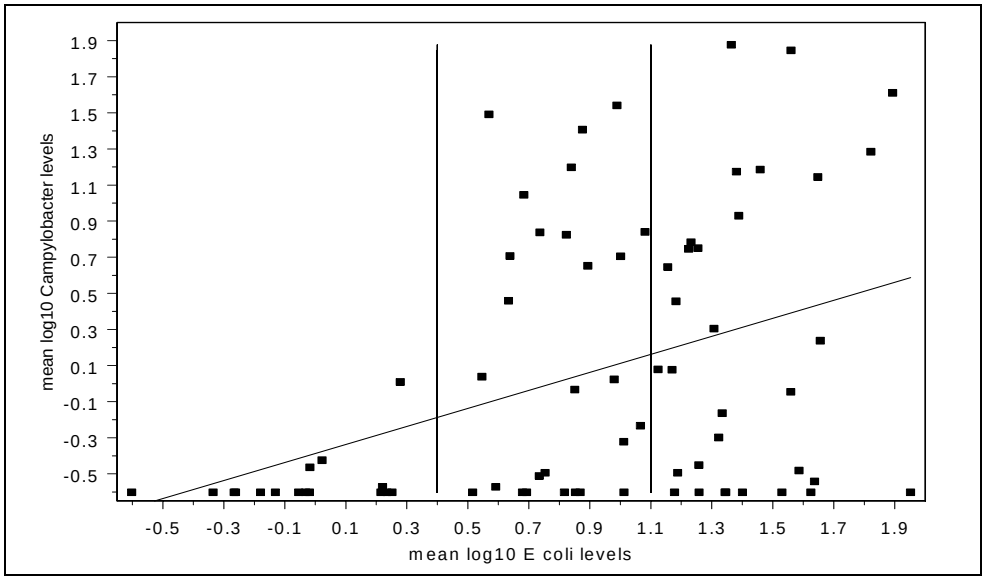

Figure 2. The 10-carcass sample set mean of $\log_{10}$ *Campylobacter* levels at post-chill versus the mean of the $\log_{10}$ *E. coli* levels at post-chill. There are 80 data points for the 20 establishments, 4 sets per establishments. For an individual sample ND, a value of -0.60 was imputed. Vertical lines are at 0.4 and 1.1 $\log_{10}$. The linear regression line is shown based on the 80 data points, and not taking establishment, season, or treatment into account.

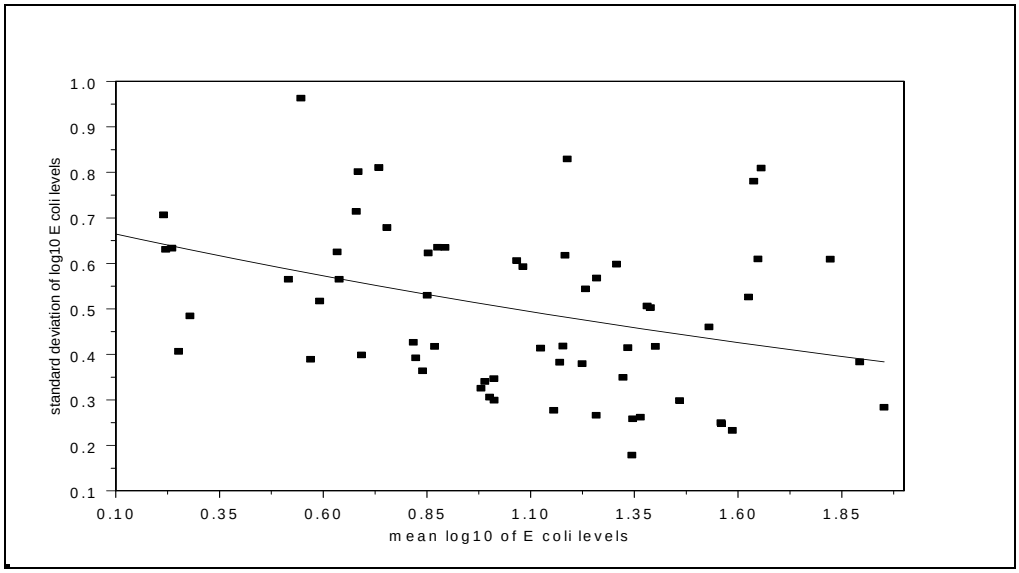

Figure 3. Plot of set-specific standard deviation of $\log_{10}$ *E. coli* levels versus mean of $\log_{10}$ *E. coli* levels. Data do not include mean values less than 0.05 $\log_{10}$ and two sets for which the standard deviations equal to 1.30 $\log_{10}$ and 1.52. Line is the predicted standard deviation derived above: $\sigma = 0.6843e^{-0.2963m}$, where "m" is the mean value.

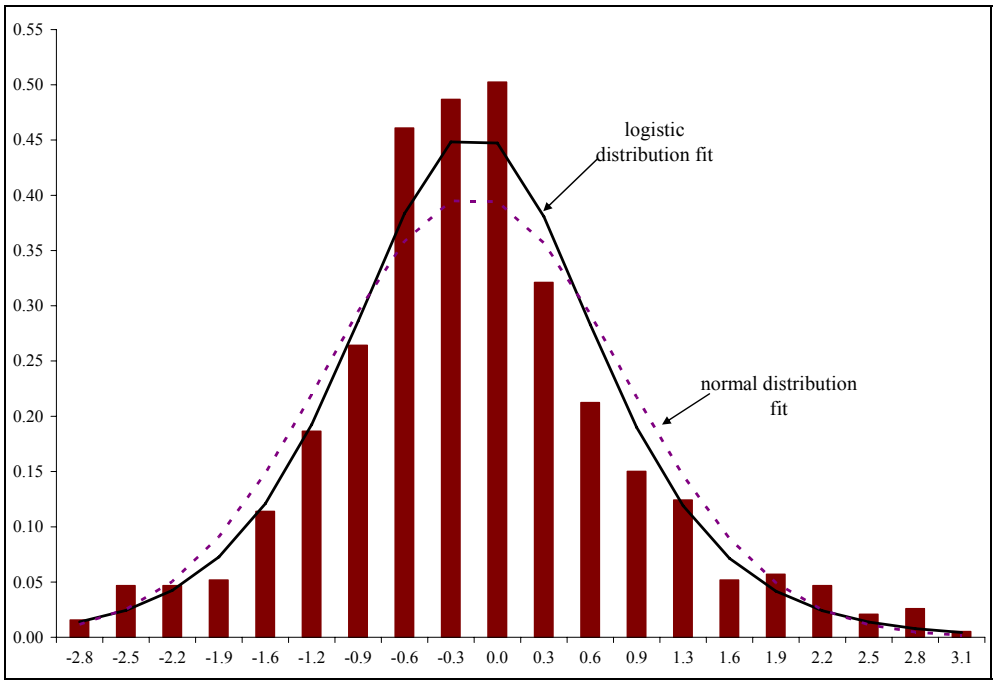

Figure 4. Histogram of standardized distribution versus fitted logistic and normal distributions at the post-chill location. Standardized values determined by subtracting from each $\log_{10}$ *E. coli* levels, the set-specific mean, and dividing by the standard deviation (actually dividing by $((n-1)/n)^{1/2}\sigma$, where is defined in Figure 3).

Re-hang

A performance standard at re-hang could encourage establishments to monitor levels of *E. coli* at different locations of processing and to use that information to help ensure that their microbial process controls are working as intended to prevent insanitary conditions. Generally speaking, higher levels of *E. coli* at re-hang will result in higher levels at post-chill (**Figure 5**). The figure shows a positive correlation between the 10-carcass-sample set-specific means of the $\log_{10}$ *E. coli* levels for re-hang and post-chill. Of particular interest is the cluster of points that occur for means at re-hang that are larger than 3.5, and means at post-chill that are larger than 1.1. This observation suggests an advisory standard of 3.5. Of the 80 sample sets at re-hang, about 40 percent of them (33) had mean values exceeding 3.5.

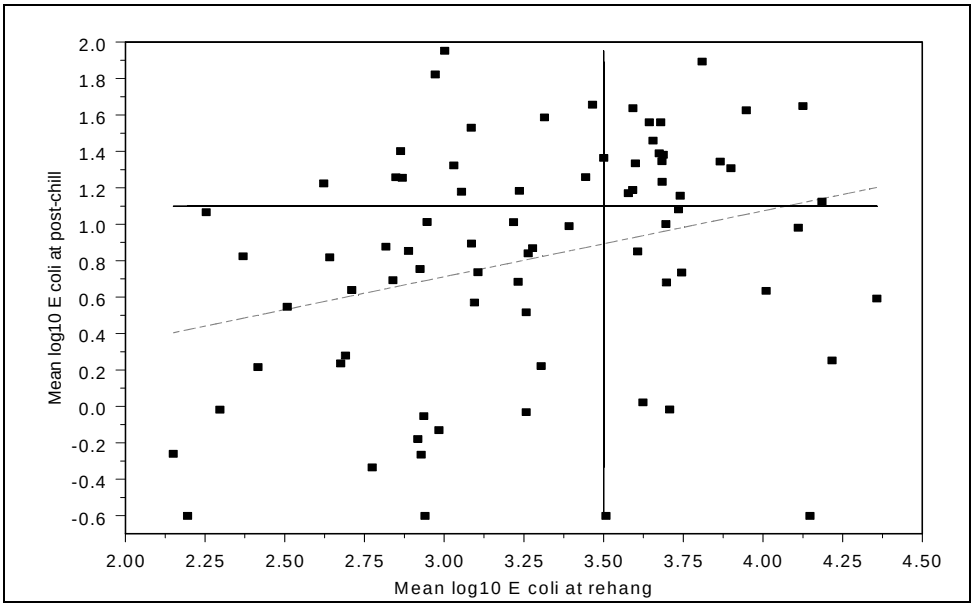

Figure 5. Plot of sample set-specific means of $\log_{10}$ *E. coli* levels at post-chill versus corresponding means at re-hang. Horizontal line is at 1.1; vertical line is at 3.5. The dotted light line is the OLS linear regression.

Campylobacter

Higher levels of *E. coli* at re-hang also seem to be associated with higher levels, or incidence of *Campylobacter* (**Figure 6**). Of particular interest, is the cluster of points that occur for means of the $\log_{10}$ *E. coli* levels that are larger than 3.5 and means of the $\log_{10}$ *Campylobacter* levels that are larger than 4, a relatively high level. The relationship of the *E. coli* levels and *Campylobacter* incidence is also apparent by noting that of the 33 sample sets that had mean $\log_{10}$ *E. coli* levels greater than 3.5, only 3 of them had less than 3 positive *Campylobacter* results (out of the 10 samples) and 26 of them (79 percent) had 9 or more positive *Campylobacter* results, while 14 of the other 47 sample sets had less than 3 positive results, and 29 of them (62 percent) had 9 or more positive results. These relationships lend support for a demarcation control limit at re-hang of a mean of the $\log_{10}$ of *E. coli* levels equal to 3.5.

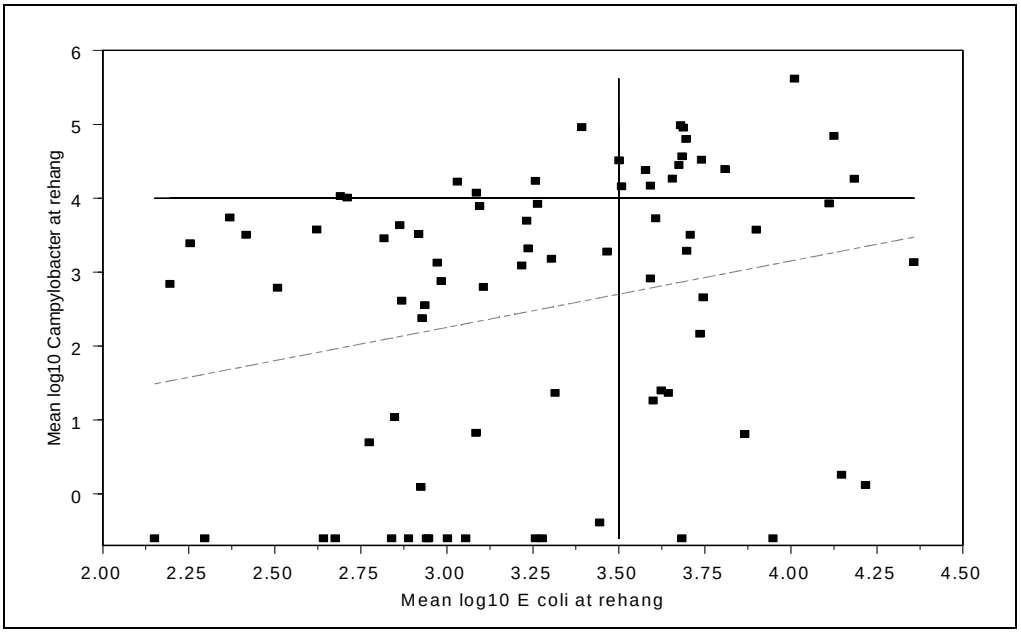

Figure 6. Plot of sample set-specific means of $\log_{10}$ *Campylobacter* levels at post-chill versus corresponding means at re-hang. Horizontal line is at 4; vertical line is at 3.5. The dotted light line is the OLS linear regression.

Salmonella

When examining all the data, no significant correlations between the mean $\log_{10}$ *E. coli* levels at re-hang and the *Salmonella* incidences at post-chill or re-hang were seen. The possibility of occurrences of high incidences of *Salmonella* at post-chill when there are high levels of *E. coli* at re-hang, regardless of the magnitude of the reduction of *E. coli* levels between the two locations, may have a theoretical explanation. *Salmonella* contamination in poultry carcasses can occur before or after evisceration. *Salmonella* that contaminates the carcass before the evisceration may tend to attach quite firmly to the skin of carcasses (Lillard 1989). In contrast, when *Salmonella* contaminate carcasses during evisceration due to alimentary tract rupture, they might be more loosely attached to skin and thus more easily removed by the washing and chilling steps than *Salmonella* that are firmly attached to the skin of live birds entering the slaughter establishment.

While there was not a perceived significant positive correlation between mean levels of the $\log_{10}$ of *E. coli* levels and *Salmonella* incidence at re-hang for these data, the possibility there would be relatively high levels of *Salmonella* if relative high levels of *E. coli* at re-hang did occur cannot be dismissed. If this were the case and if the type of attachment being discussed does not hold as strongly for *E. coli* as it might for some of the *Salmonella* cells, then the relatively high levels of *E. coli* at re-hang could be biologically related to relatively high *Salmonella* incidence at post-chill, even when there are large relative reductions of *E. coli* levels between the two locations. An examination of **Table 1**, which provides establishment-specific mean values of *Salmonella* incidence and $\log_{10}$ *E. coli* levels, where ND reported values were assigned a value of 0.25 CFU/ml, depicts possible examples of this phenomenon.

Observations that are consistent with the above phenomenon occur when there are large reductions between re-hang and post-chill of *E. coli* log levels, relatively high levels of *E. coli* at

re-hang, and at least moderate incidence of *Salmonella* at post-chill. Six establishments had mean *E. coli* levels greater than 3.5 $\log_{10}$: F, E, K, D, J, and O. At post-chill, the latter four were among the 10 establishments with *Salmonella* incidence of 0.23 or more. A conspicuous example is establishment O, which had a high mean log *E. coli* count (3.91 $\log_{10}$) at re-hang, low mean count at post-chill, and high *Salmonella* (44 percent) at post-chill.

Table 1. Summary of Establishment-specific Mean Values of *Salmonella* Incidence and Log_{10} *E. coli* Levels (data sorted by *Salmonella* incidence at post-chill)

	Salmonella		*Log E. coli*	
	Re-hang (percent)	***Post-chill (percent)***	***Re-hang***	***Post-chill***
Establishment	***Overall***		***Overall***	
F	33	3	3.6	1.52
P	35	3	3.1	0.09
C	50	5	3.38	0.7
L	93	8	3.2	-0.6
E	83	10	3.59	0.75
A	43	13	3.26	1.39
R	53	13	3.36	0.86
Q	65	15	3.17	1.33
N	75	18	2.89	0.73
M	98	20	2.55	-0.19
K	65	23	3.5	0.98
T	93	23	2.83	0.63
B	88	25	2.82	0.4
D	88	25	3.74	1.2
I	68	25	3.31	1.19
S	55	25	3.38	1.4
J	78	28	3.59	0.83
H	90	30	3.33	0.97
O	85	44	3.91	0.72
G	90	63	3.16	1.36
Mean	71	21	3.28	0.81

It is worthwhile to examine the individual sample set results for these four establishments. **Table 2** provides the mean log *E. coli* levels and *Salmonella* incidence for each set, at both re-hang (Location 1) and post-chill (Location 2). Also included is the ID number for the antimicrobial treatment that was used. The antimicrobial treatment "3" was the most effective (as discussed further below) in reducing the levels of *E. coli* and *Salmonella* incidence. However, for the results given in Table 2, the reported *Salmonella* incidence associated with this antimicrobial are relatively high when compared to the overall *Salmonella* incidence obtained when this antimicrobial was used.

Table 2. Summary of Results for Establishments D, J, K, and O

Establishment ID	Antimicrobial	Quarter	Salmonella Incidence		Mean log E. coli		Mean Reduction log E. coli
			Location 2	Location 1	Location 2	Location 1	
D	4	1	0.30	0.70	1.00	3.70	2.69
D	4	2	0.10	1.00	1.46	3.66	2.20
D	4	3	0.40	0.90	1.36	3.50	2.14
D	3	4	0.20	0.90	0.98	4.11	3.13
J	4	1	0.70	1.00	0.68	3.23	2.55
J	4	2	0.20	1.00	1.23	3.68	2.45
J	4	3	0.00	0.90	0.68	3.70	3.02
J	4	4	0.20	0.20	0.73	3.75	3.01
K	3	1	0.30	0.40	0.02	3.62	3.60
K	5	2	0.40	1.00	1.64	3.59	1.95
K	5	3	0.00	0.20	1.08	3.74	2.65
K	5	4	0.20	1.00	1.18	3.05	1.88
O	1	1	0.90	1.00	1.12	4.18	3.06
O	1	2	0.44	0.70	1.16	3.74	2.58
O	1	3	0.20	1.00	0.63	4.01	3.38
O	3	4	0.20	0.70	-0.02	3.71	3.73

A sort of counter-example to the phenomenon being discussed occurs for establishment J where, for the first quarter, *Salmonella* incidence at post-chill was high and the mean of the $\log_{10}$ measured *E. coli* levels is near the average (see Table 1). However, for the other three quarters at that establishment, the means of the $\log_{10}$ *E. coli* levels were greater than 3.5, which might be more indicative of the typical levels seen at re-hang for this establishment. A more direct counter-example is the result for this establishment in the third quarter, where the *Salmonella* incidence is 0. There is an expected variability, and the phenomenon being discussed need not happen all the time – its occurrence might depend on many factors. Over the 4 quarters though, the possibility of the phenomenon becomes apparent. As is seen, for the most part, for the data in the above table, the mean $\log_{10}$ levels of *E. coli* at re-hang exceeded 3.5.

The above discussion of the relationship of *Salmonella* incidence and mean $\log_{10}$ *E. coli* levels was not meant to provide a justification of a demarcation value of 3.5 for the mean $\log_{10}$ *E. coli* levels at re-hang. It was presented to provide a certain degree of reasonableness of a possible benefit, with respect to *Salmonella*, that might accrue as a result of processes adhering to a performance standard requiring that the mean values should not exceed 3.5. However, the nature of these data make estimating such benefit difficult, if not impossible. A better justification is seen through the relationships of mean $\log_{10}$ *E. coli* levels at re-hang with mean $\log_{10}$ *Campylobacter* levels at re-hang and mean $\log_{10}$ *E. coli* levels at post-chill, where estimates of benefits seem to be possible.

Determining the Target Distribution, F

The mean of the distribution of $\log_{10}$ of *E. coli* levels is set at 3.5. There was no correlation between the within sample set standard deviation and the sample set-specific mean of the $\log_{10}$ *E. coli* levels. An analysis of variance, after deleting ND results, and one other result that was identified as outlier (based on studied residuals from a general linear model with establishment and quarter as fixed effects equal to 4.3), leaving 795 results, yielded a standard deviation of

0.555, using MLE for an analysis of variance. **Figure 7** provides the standardized distribution, where, as with the data at post-chill, the logistic distribution fits the data better than a normal distribution.

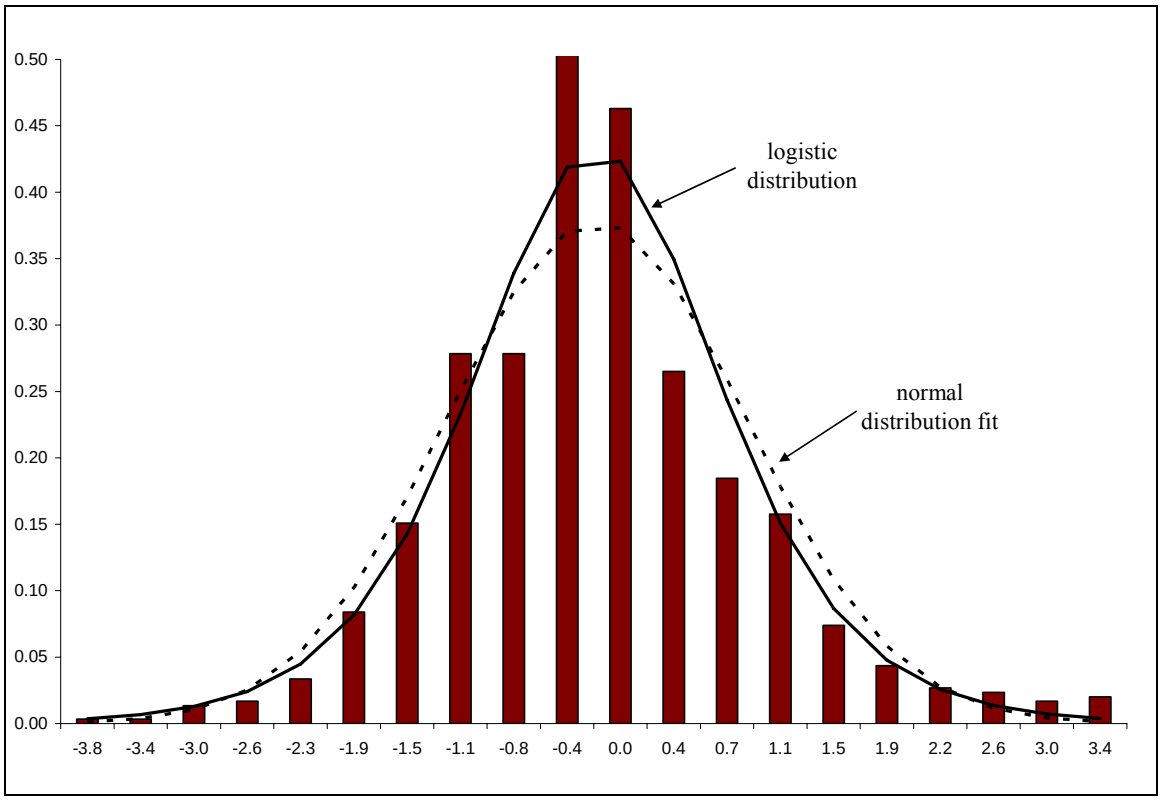

Figure 7. Histogram of standardized distribution versus fitted logistic and normal distributions at the re-hang location. Standardized values determined by subtracting from each $\log_{10}$ *E. coli* levels, the set-specific mean and dividing by the standard deviation (actually dividing by $((n-1)/n)^{1/2}\sigma$, where is defined in Figure 3).

For Reduction

The levels of *E. coli* and *Campylobacter* and the incidence of *Salmonella* decrease from the re-hang location to the post-chill location. Monitoring the reductions between two points of processing would provide assurance that controls are working properly, and provide an understanding of potential deficiencies of processing if unacceptable results for the finished product were seen. Lower than expected reductions could be due to poor processing (e.g., eviscerating) that introduce more organisms than would be expected, or that do not decrease the levels as much as expected (through the use of a specified antimicrobial). Monitoring levels and reductions throughout the system would provide information that can be used to improve the process, as well as ensure that the process controls are working properly.

The actual amount of reduction that a process needs to obtain to ensure sanitary conditions will depend on the particulars of the process. Thus, as discussed above, the reductions obtained (measured by the decrease in the $\log_{10}$ of the levels) by an establishment are presumptive with regard to sanitation, but could be used to provide a reason for further investigation. In order to achieve compliance with the performance standard at post-chill, an establishment would need to obtain a sufficient amount of reduction, which would be a factor of the levels at re-hang.

Generally speaking, the reductions of sample set-specific means of $\log_{10}$ *E. coli* levels (from re-hang to post-chill) were positively correlated with the corresponding measure of reductions of $\log_{10}$ of levels of *Campylobacter* and incidences of *Salmonella*, though the correlations are difficult to ascertain, in part, because of numerous ND values. There were 60 sets for which the incidence of *Campylobacter* (positive finding) were greater than or equal to 60 percent. Most of them had 100 percent incidence. For the 60 data points (ignoring establishment effects), the reductions of the sample set-specific means of the $\log_{10}$ of *E. coli* levels were significantly positively correlated with the reductions of *Campylobacter* incidences from re-hang to post-chill (Spearman P value = 0.0016), and with the reductions of means of $\log_{10}$ of *Campylobacter* levels (Spearman P value = 0.0039). For *Salmonella*, there were 57 data points with incidence not less than 60 percent at re-hang, and the reductions of the sample set-specific means of the $\log_{10}$ of *E. coli* levels were significantly positively correlated with the reductions of *Salmonella* incidences from re-hang to post-chill (Spearman P value = 0.030).

The public health concern here is that an establishment could have low levels of *E. coli* at re-hang, and be able to satisfy the performance standard at post-chill with a low reduction of *E. coli* levels, resulting in possible relatively high incidence or levels of pathogens, particularly if the initial incidence of levels was relatively high at re-hang. **Figures 8** and **9** provide a plot of the reduction of *Campylobacter* and *Salmonella* incidence, respectively, versus reduction of the mean $\log_{10}$ *E. coli* levels, including only those data for which the respective incidences at re-hang were not less than 60 percent. These figures suggest that to have reasonable confidence of obtaining at least a 30 percent reduction of *Campylobacter* incidence and a 60 percent reduction of *Salmonella* incidence, given relatively high incidences at re-hang, a reduction of the mean $\log_{10}$ *E. coli* levels should not be less than 2.7. Only 30 percent of the 80 sample sets had less than 2.7 mean reduction $\log_{10}$ of *E. coli* levels.

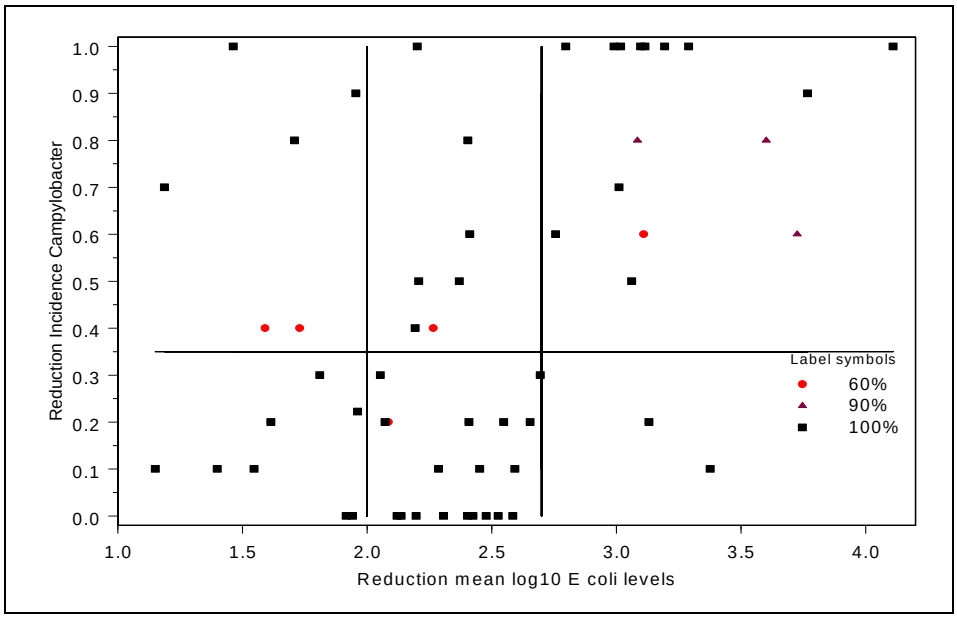

Figure 8. Plot of the Reduction of *Campylobacter* Incidence Versus Reduction of the Mean $\log_{10}$ *E. coli* Levels, Including Only those Sample Sets for which the *Campylobacter* Incidence at Re-hang was not Less than 60 Percent (60 points). Symbols indicate percent number of positive samples. Vertical lines at 2.0 and 2.7 $\log_{10}$, horizontal line at 0.35. Twenty-nine data points have an incidence reduction of less than 0.35.

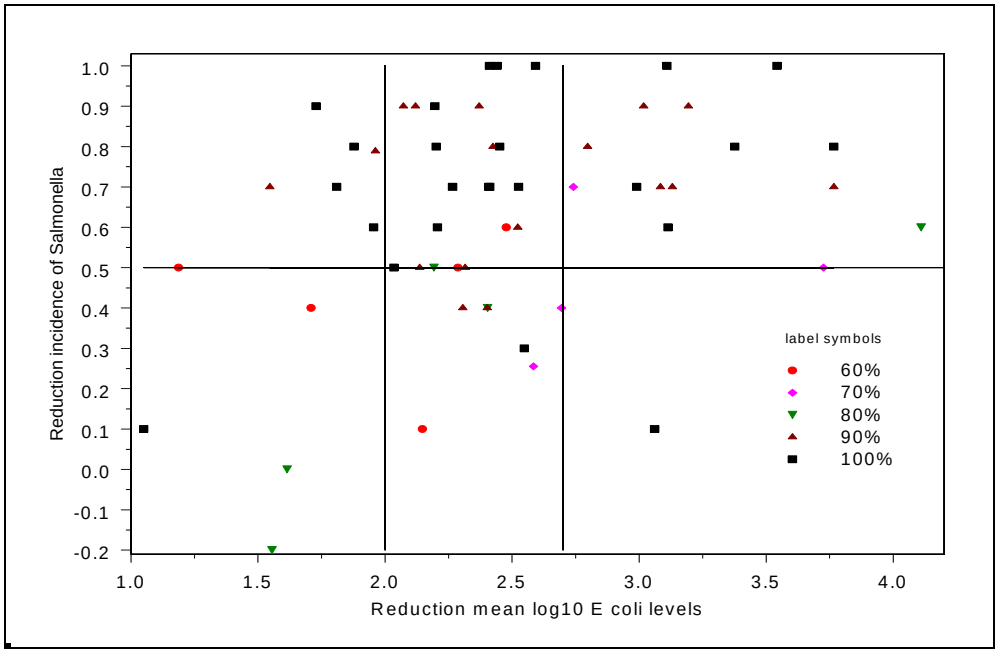

Figure 9. Plot of the Reduction of *Salmonella* Incidence Versus Reduction of the Mean Log_{10} *E. coli* Levels, Including Only those Sample Sets for which the *Salmonella* Incidence at Re-hang was Not Less than 60 Percent (57 points). Vertical lines at 2.0 and 2.7 log_{10}, horizontal line at 0.5. One data point not shown, with x-axis value > 4 and y-axis value = 1.

Figure 8 shows a greater likelihood of a low reduction of *Campylobacte*r incidence when the reduction of mean log_{10} *E. coli* levels is not greater than 2.0 log_{10}. From the 60 data points shown in Figure 8, 14 had less than a 2.0-log_{10} mean reduction of *E. coli* levels and, of these, 8 had less than 0.35 reduction of *Campylobacter* incidence. For *Salmonella* (Figure 9), of the 57 data points, 11 had the reduction of mean log_{10} of *E. coli* levels not greater than 2.0 log_{10} and, of these, 5 had less than a 0.5 reduction of *Salmonella* incidence.

As a consequence of the above type of considerations, the advisory performance standard of mean log_{10} reduction might be set equal to 2.0. From the 80 sample sets, 19 of them, almost 25 percent, had mean reductions less than 2.0 log_{10}.

Potential Concerns

The ARS data was based on 100-ml rinse rather than the usual FSIS 400-ml rinse same that has been used for its baseline surveys. Also, the ARS data indicated a potential seasonal effect. In addition, there is interest regarding the correlations between the *E. coli* levels and *Salmonella* incidence and *Campylobacter* levels. Below are brief discussions of these issues.

Correlations or Relationships Between E. coli Levels and Pathogens – Antimicrobial Treatments

Figures 1 and 2 show relationships that suggest that associated with higher levels of *E. coli*, there is a greater likelihood of higher incidence of *Salmonella* and higher levels of *Campylobacter*. The observed relationship does not imply that a cause and effect relationship, as estimated from a model based on the observed relationship, can be assumed wherein the change of levels of *E. coli* would cause a corresponding change of *Campylobacter* levels. However, a possible cause and

effect relationship might be estimated based on antimicrobial use, since such usage does offer an explanation the observed relationships.

Furthermore, significant correlations for the most part exist between *E. coli* levels and *Salmonella* incidence, and *Campylobacter* levels within the 10-carcass-rinse-sample-sets.

While there is a tendency for a positive trend of *Salmonella* incidence with increasing means of log *E. coli* levels that is not statistically significant by usual criteria (Figure 1), there is also seen an increased variation with increasing levels. These can be described by the following models:

Model 1: x = mean of $\log_{10}$ *E. coli* levels.

$\text{logit}(p) = \alpha_0 + \alpha_1 (x-0.8) + e_w$

$e_w \sim \text{normal}(0, \sigma_w)$, where $\sigma_w^2 = \beta e^{2\exp(\rho)(x+0.60)}$

m ~ binomial (p,n), where m is the number of positive results in the set, and n is the number of samples (= 10) within the set,

where α_0, α_1, β, ρ are parameters.

(Ignoring establishment effect)

The goodness of fit statistic is:

$L_1 = -2\text{logLik} = 301.0$.

Model 2 is the same as model 1, except excluding heteroscedasticity variance assumption: σ_w is constant, so there are only three parameters: α_0, α_1, and σ_w.

$L_2 = -2\text{logLik} = 307.5$.

The difference $L_2 - L_1 = 6.5$ is statistically significant at the 0.01 level, based on the chi-square distribution approximation of the distribution of the difference with 1 degree of freedom. Thus model 1 is "better" than model 2.

Model 1a includes between plant-variance effect, and heteroscedasticity assumption for the within plant, between sample set standard deviation. Estimates were derived using WinBugs1.4.

$\text{logit}(p) = \alpha_0 + \alpha_1 x + e_p + e_w$

$e_p \sim \text{normal}(0, \sigma_p)$, where σ_p = standard deviation is assumed constant,

$e_w \sim \text{normal}(0, \sigma_w)$, where $\sigma_w^2 = \beta e^{2\exp(\rho)(x+0.60)}$

Five parameters: α_0, α_1, β, ρ, σ_p. The two-sided significance of the slope α_1 was 0.24.

The above models quantify in some sense the described tendencies of the observed data given in Figure 1. They are not causal models, where predictions of changes of the *Salmonella* incidence could be made based on assumed changes in *E. coli* levels. What is observed is the general increase of high levels of *Campylobacter* with increased log-level of *E. coli*. Analysis with the logit of the *Campylobacter* incidence gives similar results as above for *Salmonella*.

Causes for lower levels of *E. coli* and *Campylobacter* and incidence of *Salmonella* could be due to the antimicrobial treatment used. The purpose of this document is not to provide a thorough

presentation of the possible impact of antimicrobial treatments, or other types of interventions. Such analyses will be given in the risk assessment. However, a brief presentation of summary data might be of some interest.

Antimicrobial treatments were divided into three categories: A; none or ineffectual (with respect to reduction of *E. coli* levels [numbers 0 and 6 in Table 1]; B. typical; and C special (which provided the greatest reduction of *E. coli* [number 3 in Table 1]).

Tables 3 and **4** present more detailed summaries using the above categories. What is clear here is the apparent impact of the special treatment on the levels and incidence of the organisms.

Table 3: Summary of Results by Specific Antimicrobial Treatment (all data)
(The treatment without an antimicrobial is labeled 0)

ID Number for Treatment	*Number of Sets*	*Mean Log E. Coli*		*Salmonella Incidence*		*Mean Reduction Log E. Coli*
		Location 1	*Location 2*	*Location 2*	*Location 2*	
6	4	3.26	1.39	1.87	0.13	-0.30
0 [a]	8	3.38	1.44	1.93	0.33	0.35
2	10	2.87	0.24	2.63	0.20	-0.22
5	13	3.33	0.97	2.36	0.14	-0.04
4	25	3.24	0.98	2.26	0.23	0.56
1	7	3.63	1.22	2.42	0.36	0.18
3	13	3.40	0.00	3.40	0.10	-0.33
	80	3.28	0.81	2.47	0.21	0.12

[a] No treatment.

Table 4. Summary of *Salmonella* Incidence and *E. coli* Results (entries are mean values)

Treatment (# obs)	*Incidence Post-chill (percent)*	*Incidence Re-hang (percent)*	*Reduction of Incidence (percent)*	*Log E. coli Post-chill*	*Reduction Log E. coli*
A (12)	25.8	55.0	29.2	1.43	1.91
B (55)	22.1	75.6	53.5	0.873	2.37
C (13)	10.0	66.9	56.9	0.0028	3.40
All (80)	20.7	71.1	50.4	0.814	2.47

Table 5. Summary of *Campylobacter* Incidence and *E. coli* Levels

Treatment (# obs)	*Incidence Post-chill (percent)*	*Incidence Re-hang (percent)*	*Reduction Incidence (percent)*	*Log E. coli Post-chill*	*Reduction Log E. coli*	*Log Campy Post-chill*	*Log Campy Re-hang*	*Reduction Log Campy*
A (12)	38.3	64.2	25.8	1.43	1.91	0.05	1.95	1.90
B (55)	44.5	75.8	31.1	0.873	2.37	0.13	2.67	2.54
C (13)	13.1	79.2	66.1	0.0028	3.40	-0.48	2.32	2.81
All (80)	38.5	74.6	36.2	0.814	2.47	0.02	2.51	2.49

Correlations within 10-carcass Sample Sets

The results within each of the 80 sample sets can be thought of as being measured levels or incidences on carcasses that have been processed under the same conditions. In this sense, the data from the 80 sample sets could be thought of as data collected from 80 "controlled" experiments, so that relationships within these 80 sample sets represents those that are unencumbered by confounding factors.

At post-chill, of the eighty 10-carcass sets collected, a within-set correlation could be computed between sample-specific *Salmonella* incidences and *E. coli* levels over the samples for 49 sets. The mean Spearman correlation was 0.11, with 31 positive correlations and 18 negative correlations (P-value = 0.02 for the signed-rank test; P-value = 0.09 for the sign test). In a similar fashion, for *Campylobacter* and *E. coli* levels, the mean Spearman correlation was 0.12, with 29 positive correlations, 18 negative correlations, and 2 zero correlations (P-value=0.04 for the signed-rank test; P-value = 0.14 for the sign test).

At re-hang, from 50 within-set correlations that were computed between the incidence of *Salmonella* and *E. coli* levels, the mean Spearman correlation was 0.078, with 28 positive correlations and 21 negative correlations, with a significant signed-rank test (P-value = 0.13) and significant sign test (P-value = 0.39). For *Campylobacter* levels, the mean of 66 correlations was 0.33, with 54 positive correlations and 12 negative correlations, with a significant signed rank test (P-value < 0.001) and significant sign test (P-value < 0.001).

The "strongest" correlation occurs for *Campylobacter* and *E. coli* levels at re-hang. At post-chill, the strength of the correlation would dissipate some due to the mixing of carcasses within the chiller tank. Even so, there was a significant positive correlation at post-chill. The significant correlations within the 10-carcass samples between the $\log_{10}$ levels of *E. coli* and *Campylobacter* suggest the possibility of a direct relationship between these two levels on individual carcasses that might be considered usable in a causal model. In other words, suppose for some reason such as improved husbandry practices or improved processing (feeding, shipping, etc.), there was a slight reduction of *E. coli* levels on carcasses, as measured at re-hang. What could be said of the corresponding impact on levels of *Campylobacter* for carcasses subjected to the same treatments and environments? This relationship was explored by performing linear, mixed effects, regressions of the of the *Campylobacter* levels (dependant variable) versus the $\log_{10}$ of the *E. coli* levels (as the independent variable), with the 10-carcass sample sets considered as a random "subject" factor and assuming the slope and intercept are distributed as a bi-normal distribution. At re-hang, there were fifty-two 10-carcass sample sets for which there were no ND *Campylobacter* measured values. With one exception, the distribution of the set-specific slopes and intercepts "looked" nearly normal. Excluding the data from the exceptional set, from the mixed effect regression, the estimated expected value of the slope was 0.540, with a standard error equal to 0.064. The estimated standard deviation of the slope was 0.267, with standard error of 0.071. Thus, ignoring the uncertainty of the estimated parameters, a 90 percent probability interval for the slope range would be (0.100, 0.980). A slope of 0.540 would imply that, for a 50 percent reduction of *E. coli* levels at re-hang (amounting only to a 0.3-$\log_{10}$ decrease), there would be about a 31-percent reduction of *Campylobacter* levels (with a standard error of about 3 percent). Thus, a *Campylobacter* log reduction of slightly more than 0.1-$\log_{10}$.

A 0.1-$\log_{10}$ reduction of *Campylobacter* could be a significant reduction regarding public health impacts. For example, dose-response curves to model illness from ingesting *Campylobacter*, have been based on one-hit models: p(d) = 1- exp(-rd), where d is the dose, and r is a parameter (Teunis et al. 2005). Thus, if this dose-response model were approximately correct, significant human health benefits might be realized if processes reduce *Campylobacter* levels, even by what might be considered small amounts (a reduction of 0.1 $\log_{10}$ of pathogen levels at re-hang could translate to a predicted 26 percent reduction of illnesses, everything else being equal). The risk assessment will address these issues in detail.

Seasonality

Table 6 provides means of the $\log_{10}$ *E. coli* and *Campylobacter* levels and the incidence of *Salmonella* computed over the 4 quarters of data collection, and over the whole study.

Table 6. Mean $\log_{10}$ *E. coli* and *Campylobacter* Levels and *Salmonella* Incidence for Re-hang and Post-chill 100-ml Broiler Rinses, by Season of Collection, and Overall 100-ml Broiler Rinses, by Season of Collection, and Overall

	Re-hang				*Post-chill*			
	Log E. coli		*Salmonella Positive Mean (percent)*	*Log Campylobacter Mean*	*Log E. coli*		*Sal Mean (percent)*	*Log Campylobacter Mean*
Quarter	*No.*	*Mean*			*No.*	*Mean*		
Autumn*	200	3.26	72	2.72	200	0.71	29	-0.05
Winter	200	3.24	76	2.26	199	0.97	20	0.01
Spring	200	3.32	70	2.73	200	0.92	17	0.35
Summer	200	3.32	74	2.32	199	0.65	18	-0.23
All	**800**	**3.28**	**71**	**2.51**	**798**	**0.81**	**21**	**0.02**

* In the initial quarter of the study, broiler rinses from five establishments were rejected due to temperature control. Broiler rinses were collected in these establishments again 12 months later. Seasonal relationships for *E. coli* levels held for these five establishments, on average, and are thus not shown separately.

The mean values of the $\log_{10}$ *E. coli* and $\log_{10}$ *Campylobacter* levels at post-chill over the winter and spring quarters (2^{nd} and 3^{rd} quarters of the survey) were larger than the corresponding mean values over the summer and fall quarters, both by nearly 0.3 $\log_{10}$. At re-hang, the means of the $\log_{10}$ *E. coli* and $\log_{10}$ *Campylobacter* results did not display significant seasonality. However, the apparent seasonality effect at post-chill noted above could in part be explained by the confounding of season and the antimicrobial chemical or the chiller water acidification treatments that were applied during the study. Some establishments changed treatments in the course of the study, creating in them a confounding of the season and treatment effects. Regarding the effect of acidified chiller water treatment, there were eight establishments that did not have the same acidified chiller water treatment throughout the study and did not use antimicrobial treatment B. An analysis of variance with the mean reduction of $\log_{10}$ *E. coli* levels as the dependent variable, accounting establishment effects, did not indicate statistical significance of water acidification (P value = 0.16), though the mean reduction in the $\log_{10}$ *E. coli* levels was greater by 0.31 when the chiller water was acidified.

Thus, a (partial) confounding of treatments with season could be created because most establishments (15) changed treatments during the course of the survey.

From this perusal of the data, as discussed above (Table 3), three antimicrobial chemical treatment classes of data were identified for descriptive and analysis purposes: one class consisted of data for which the treatment was not applied or the treatment was the antimicrobial chemical treatment A, identified above; the second class consisted of data for which antimicrobial chemical treatment B was applied; and the third class was the remainder.

Mixed linear effect models were performed with dependent variables equal to the 10-carcass sample set-specific mean of $\log_{10}$ *E. coli* levels, and including a quarter effect among the independent variables, deleting any establishment that used antimicrobial treatment B (six establishments); assuming a random establishment effect, and including the mean of $\log_{10}$ *E. coli* levels at re-hang as a covariate. One additional observation was deleted as an outlier that had a studentized residual exceeding 3.4 in absolute value (the next largest values were close to 2). Thus the number of observations (sample sets) in the model was 55. The factor of acidified chiller water usage was not statistically significant when included in the model, and was not used. For the mean of $\log_{10}$ *E. coli* levels, the estimated season effect (the mean for the winter-spring minus the mean for the summer – fall) was 0.217, (P value = 0.01, Scheffé's multiple comparison P value = 0.07, based on 3 and 37 degrees of freedom for the F-statistic). When the covariate was excluded, the P value was 0.04, and the Scheffé's multiple comparison P value = 0.23.

Clearly, 1 year of data cannot establish seasonality, but in any case, these data suggest a possible effect of time of the year related to season, at post-chill, but not at re-hang. The reason for this is not clear. It is possible that for some reason the levels within the intestines of young chickens are greater during some parts of the year and thus this would cause higher levels at post-chill but not at re-hang. In any case, the implication is that improved process control between re-hang and post-chill would be needed to maintain a constant outgoing product quality and maintain a constant probability passing the compliance criteria for maintaining sanitary conditions with respect to *E. coli* levels, regardless of the time of year of sampling.

400-ml Versus 100-ml Rinse Relationship

For its baseline surveys, FSIS has collected 400-ml rinse samples, and the present requirement for *E. coli* levels in the HACCP/Pathogen Reduction rule is based on 400-ml rinse samples. The ARS data from which the above described nonpresumptive generic *E. coli* performance standard is derived is based on 100-ml rinse samples. Since most of the FSIS historical data is based on 400-ml rinse samples and there is a need to determine the potential impact (cost and benefits) of the new performance standards, it is perhaps necessary to have some knowledge of the relationship between results that are obtained from rinse samples of different sizes. Furthermore, since sampling for other pathogens is typically based on 400-ml rinse samples, an answer to the question of whether or not the *E. coli* performance standards derived from the ARS study data could be expressed in terms of 400-ml rinse sample, or whether or not pathogen related performance standards based on 400-ml rinse samples could be expressed in terms of 100-ml rinse samples would be important insofar as this could lead to a more efficient sampling program by eliminating the need for separate 100- or 400-ml rinse samples. Without such a conversion relationship, the establishments and FSIS would need to use 100-ml rinse samples for determining compliance with the *E. coli* performance standards, while sampling for *Salmonella* or other pathogens would continue with 400-ml rinse samples, since the present performance

standard for *Salmonella* is based on 400-ml rinse samples, and possible future performance standards derived from FSIS baseline data would be based on 400-ml rinse samples.

There are at least two primary factors that could affect the comparison of results for 100-ml and 400-ml rinse samples: (1) differential numbers of cells being pulled or washed off the carcasses; and (2) different antimicrobial concentrations in the samples differentially affecting the recovery of cells. For the latter, the 100-ml rinse would have a higher concentration of antimicrobial residual in the sample; and, thus, since the sample is not analyzed until the next day, would lead to a greater reduction of numbers of recovered cells compared to levels when the sample was collected for the 100-ml rinse.

For the first factor, there is no a-priori reason to believe which way the impact would be, unless it is believed for some reason, for example, that the 400-ml rinse would not wash off more than 4 times as many cells as the 100-ml, in which case the 100-ml samples would provide higher levels, on average. Otherwise, a-priori it is possible that the 400-ml rinse washes off more or less than 4 times the number of cells than the 100-ml rinse, so there is no reason a-priori to think that one or the other would provide higher measured levels.

To obtain information of possible relationships between results obtained on 100-ml and 400-ml rinse samples, FSIS, with ARS analyzing the samples, conducted a small, 3-day study at one establishment. For each day, 50 pairs of "matched" samples were collected, for a total of 300 samples (150 of 100-ml rinse and 150 of 400-ml rinse). For each sample, two 1-ml portions of the rinse sample were analyzed for *E. coli* levels. For *Salmonella*, 30 ml of the sample was analyzed for its presence. For Campylobacter, four 0.25-ml portions were analyzed, but it is not known how these were selected from the rinse samples. Over the 3 days, carcasses of birds from 5 growers were sampled, covering 18 poultry houses. The matching was obtained in sets of 5 rinse samples from carcasses for each size. That is, at a given time, 10 carcasses were sampled; 5 using 100-ml rinses, and 5 using 400-ml rinses. There were 30 sets, 10 each day. It is assumed that within a set of 5 samples, the results are independent. The average of the reported *E. coli* plate levels (CFU/ml) was computed for each sample. This is called the sample level.

Table 7 provides a summary of the results, by day of sampling and grower. Fifty matched samples per day; one 100-ml sample was not analyzed from day 1. The second to last column is the $\log_{10}$ of the ratio of the average sample levels for the 400-ml samples versus that of the 100-ml samples. For the last column, for reported ND results, a value of 0.25 CFU/ml was imputed.

Table 7. Summary of Comparison of Results for 400-ml and 100-ml Rinse Samples

Day	*Grower*									
		N	*100 ml*	*400 ml*	*100 ml*	*400 ml*	*100 ml*	*400 ml*	*100 ml*	*400-100*
1	1	25	2_0.0	20.0	10	0	72.0	100.0	0.03	0.59
1	2	10	11.1	20.0	0	0	33.3	80.0	0.99	0.83
1	3	15	13.3	0.0	1	0	26.7	80.0	1.19	0.92
2	3	20	35.0	25.0	11	1	95.0	100.0	-0.08	-0.01
2	4	30	20.0	33.3	1	0	46.7	83.3	0.59	0.55
3*	4	10	0.0	0.0	0	0	20.0	90.0	0.94	1.26
3	5	40	17.5	42.5	2	1	82.5	100.0	0.26	0.40
Pooled		150	18.8	26.0	25	2	62.4	92.7	0.23	0.54

*One set had all 100-ml rinse sample results reported as ND.

The results of the one set referred to in the above table are:

Observation	*Day*	*Grower*	*House*	*Set*	*Rinse Sample*	*Count 1*	*Count 2*
1	3	4	1	10	100	0	0
2	3	4	1	10	100	0	0
3	3	4	1	10	100	0	0
4	3	4	1	10	100	0	0
5	3	4	1	10	100	0	0
6	3	4	1	10	400	80	40
7	3	4	1	10	400	8	4
8	3	4	1	10	400	16	20
9	3	4	1	10	400	6	4
10	3	4	1	10	400	2	2

Based on the results given in Table 7, *E. coli* incidence and measured levels were generally higher for the 400-ml rinse samples, but for *Campylobacter* the reverse trend was quite evident. The *Campylobacter* levels were generally low and there seemed to be a grower effect, at least based on the 100-ml rinse samples. For the two positive *Campylobacter* results for the 400-ml, the $\log_{10}$ *Campylobacter* levels were 0.70 and 1.08. For the 25 *Campylobacter* positive 100-ml samples, there were 6 samples with a count of 1 cell in the plate counts, and only 2 with more than 10 cells (1.08 and 1.38 log).

For *Salmonella* incidence, the comparisons were ambiguous. For the most part, the incidences for the two types of samples were similar. However, for the 5th grower on the third day, the incidence for the 400-ml rinse samples was more than twice that for the 100-ml rinse samples (43 percent versus 18 percent).

Regarding *E. coli*, it is evident that the measured levels are greater within the 400-ml rinse samples than within 100-ml rinse samples. Comparative values of selected percentiles are given below.

Table 8. Selected Percentiles for Log_{10} Measured *E. coli* Levels

Volume	*Median*	*75th*	*90th*	*95th*
100 ml	0.544	1.24	1.78	1.88
400 ml	1.079	1.52	1.95	2.18

For *E. coli*, is there a constant relationship, over the range of measured levels, between the results obtained for the 100-ml rinse samples and the 400-ml rinse samples? More generally, can a conversion factor be developed that would relate results obtained for 100-ml to 400-ml rinse samples. Models for determining these questions could be constructed as follows:

If x is the number of cells for a carcass, then the measured results can be modeled through three stages:

1. The rinse sample pulls off (recoveries) an expected certain percentage of them, say p.

2. The 1-ml sample used to analyze them would be expected to have px divided by the size of the rinse.

3. If serial dilutions were performed, then the expected value of the counted cells (CFUs) would decreases accordingly.

It is assumed that the number of cells is the number of CFUs that were counted.

The simplest model would be to assume that the distribution of the cells within the rinse solution is uniform and the sum of the counts from the plates for a sample is distributed as a Poisson distribution. That this assumption might not be strictly true is seen from some results listed below, where C1 and C2 are the two plate counts reported for the sample. It is assumed that these were not diluted, that is, the counts represent the counts obtained for the 1-ml plates.

Sample Number	*Day*	*Rinse Size*	*C1*	*C2*
220	2	400	50	80
221	3	400	22	44
222	3	100	12	2
223	3	400	26	50
224	3	400	5	19
225	3	400	111	69
226	3	400	100	60
227	1	400	30	60
228	1	400	160	20
229	1	400	80	40
230	3	400	80	40
231	3	400	51	97
232	3	100	29	6

Most of the results given above are for the 400-ml rinse, which might indicate that the degree on heterogeneity might be greater for the 400-ml rinse samples compared to 100-ml rinse samples. A second point is the seemingly inordinate number of results that are multiples of 10. It is possible that estimated counts were rounded for some samples. It is also possible that some of the results were obtained using serial dilutions. For example, the result 111 could be obtained by counting 107 CFU on a 1-ml sample, and 15 on a 0.1-ml sample.

Model 1: For each set, there are 10 samples, 5 with 100-ml rinses, and 5 with 400-ml rinse (with one exception). Assume for the moment the simplest model. For each set, it was assumed that the recovery was p_1 for the 100-ml rinse, and p_4 for the 400-ml rinse, and that x is distributed a log normal, with parameters, μ and σ. The recovery factors include possible die-off due to the antimicrobial concentrations that might impact recovery. The statistic of interest is: $Lf = \log_{10}(p_4/p_1)$. Without loss of generality, in the following, p_4 was assumed to be equal to 0.5 (changing this value does not significantly affect the following estimates). Estimates of parameter values were computed using SAS 9.1, the non-linear and linear mixed effect procedures.

Figure 10 is a plot of the estimates of Lf versus μ for the 29 sets (excluding the one set for which all five 100-ml rinse samples were reported as ND).

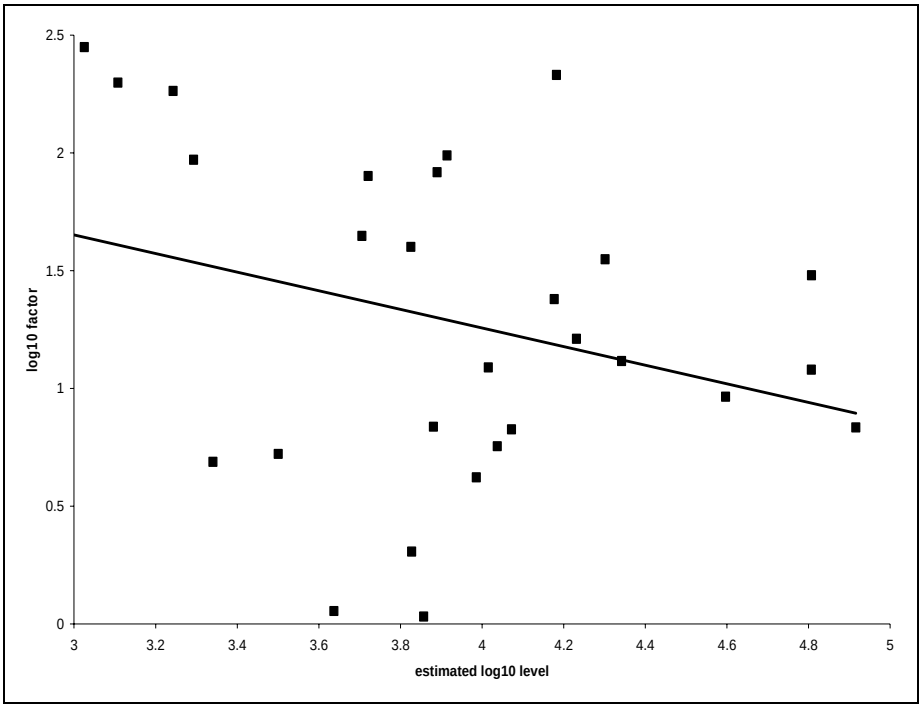

Figure 10. Plot of the estimates of Lf versus μ for the 29 sets (excluding the one set for which all five 100-ml rinse samples were reported as ND), together with linear regression line.

If "house within grower within day" (18 distinct houses) is considered a random factor, then the slope of the linear regression of Lf versus μ is not significant with two-sided P value = 0.25. Assuming there is no relationship, and treating "house within grower" as a random factor, the estimated mean of Lf is 1.33, with a standard error of 0.142, resulting in a 95 percent confidence interval for the true mean of (1.03, 1.63) based on 17 degrees of freedom. Subtracting $\log_{10}(4)$, to account for the difference of the rinse sample sizes, yields an estimate of the mean difference for $\log_{10}$ levels of 0.73 $\log_{10}$, with a 95 percent confidence interval of (0.43, 1.03).

Combining all data, except deleting the data from the set identified in Table 4, yields an estimated mean difference for $\log_{10}$ levels of 0.58 $\log_{10}$, with a 95 percent confidence interval of (0.35, 0.83). In another analysis, based on individual values of $\log_{10}$ *E. coli* levels, using the imputed value of 0.25 CFU/ml for ND reported values, and deleting the data from the set identified in Table 4, a linear mixed model, with "house within grower" a random factor yields an estimated mean difference for $\log_{10}$ levels of 0.54 $\log_{10}$, with a 95 percent confidence interval of (0.31, 0.77).

The lack of significance for a linear relationship of Lf versus μ does not eliminate the possibility that some type of relationship actually exists. The relatively large confidence intervals also preclude selecting a value to use for converting results obtained for 100-ml rinse samples to results that would have been obtained if 400-ml rinse samples had been used. More research is needed before a reasonably accurate relationship can be developed for regulatory application.

ATTACHMENT 1: MATERIAL AND METHODS OF THE ARS STUDY

Sampling

All 127 large (i.e., 500 or more employees) United States Department of Agriculture (USDA) federal-inspected young chicken slaughter establishments in operation in autumn 2004 were eligible for the study. A random sample of 20 establishments (about 1 in 6) was selected. Every 3 months, FSIS personnel collected 10 broiler-carcass 100-ml rinse samples at re-hang (post-pick) and post-chill from the same flock. Each carcass was placed in a sterile plastic bag and 100-ml of buffered peptone water (Solar Biologicals, Ogdensburg, NY) was added. The collector shook the bag by hand for 1 minute, removed the carcass and asceptically collected the rinse in a snap top vial, which was refrigerated, packaged, and shipped to the Agricultural Research Service (ARS) Bacterial Epidemiology and Antimicrobial Resistance Laboratory in Athens, GA, on freezer packs by overnight courier. Rinse temperature was monitored after receipt in the laboratory. In the first quarter of the study, rinse samples from five establishments were discarded because they were received at the laboratory at or above 10°C. In these establishments, FSIS personnel collected rinses again 1 year later to provide data for them for all four quarters.

Microbiology

Generic E. coli

E. coli were enumerated by inoculating serial dilution of rinses onto *E. coli* Petrifilms (3M Corporation, St. Paul, MN). Sterile saline (in 0.85 percent) was used for dilution. After incubation at 35ºC for 24 hrs, typical *E. coli* colonies were counted.

Campylobacter

Levels (CFU/ml) of *Campylobacter* were estimated by direct plating serial dilutions of carcass rinse on 11 Campy Cefex agar plates. In the second, third, and forth quarters of the study, in order to improve sensitivity of detection for low levels at post-chill, four 0.25-ml aliquots of undiluted rinse were plated onto four agar plates. Plates were incubated at 42°C under microaerophilic atmospheric conditions: 5 percent O_2, 10 percent CO_2, and 85 percent N_2. Wet mounts of presumptive *Campylobacter* colonies were examined by phase contrast microscopy and latex bead agglutination testing (Microgen Bioproducts Ltd, Camberley, Surrey, UK).

Salmonella

Testing for *Salmonella* used standard FSIS methods for isolation from 12 poultry rinses. One ml of a 30-ml aliquot of each young chicken rinse was added to sterile buffered peptone water and incubated at 35+2°C for 20 to 24 hours. Gene amplification (BAX®, E. I. du Pont de Nemours and Company, Wilmington, DE) was conducted on lysed cells following enrichment. PCR positive rinses were plated, and isolates were biochemically and serologically confirmed.

635 *Statistics*

636 Statistical analyses were performed on the $\log_{10}$ of the average measured levels of duplicate
637 plates. ND results (no cells counted on either plate for a sample) for *Campylobacter* or *E. coli*
638 were set to 0.25 CFU/ml or ½ the limit of detection, 0.5 CFU/ml. For a few samples, large
639 discrepancies were seen between levels on duplicate plates (e.g., one plate had no cells and
640 another had ≥ 200 CFU/ml). In such cases, the no cell result was deleted. For *E. coli*, this
641 occurred 7 times in 1,598 samples (< 0.5 percent). The same rule was used to estimate levels of
642 *Campylobacter*, resulting in 6 adjustments, all at the re-hang location. Outliers were identified
643 by graphical analysis or by examining studentized residuals. In order for data to be deleted as an
644 outlier, the absolute studentized residuals had to be greater than 3.5 ($P<0.0005$).

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