Microbial Concentrations on Fresh Produce Are Affected by Postharvest Processing, Importation, and Season

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ABSTRACT

In the United States, the proportion of foodborne illness outbreaks associated with consumption of contaminated domestic and imported fresh fruits and vegetables (produce) has increased over the past several decades. To address this public health concern, the goal of this work was to identify and quantify factors associated with microbial contamination of produce in pre- and postharvest phases of the farm-to-fork continuum. From 2000 to 2003, we collected 923 samples of 14 types of produce (grown in the southern United States or in the northern border states of Mexico) from 15 farms and eight packing sheds located in the southern United States. To assess microbial quality, samples were enumerated for *Escherichia coli,* total aerobic bacteria, total coliforms, and total *Enterococcus.* Most produce types had significantly higher microbial concentrations when sampled at the packing shed than when sampled at the farm. In addition, we observed seasonal differences in the microbial concentrations on samples grown in the United States, with higher mean indicator concentrations detected in the fall (September, October, and November). We developed a predictive, multivariate logistic regression model to identify and quantify factors that were associated with detectable concentrations of *E. coli* contamination on produce. These factors included produce type (specifically, cabbage or cantaloupe), season of collection (harvested in the fall), and packing step (bin, box, conveyor belt, or turntable). These results can be used to identify specific mechanisms of produce contamination and propose interventions that may decrease the likelihood of produce-associated illness.

Over the past decades, the proportion of foodborne disease outbreaks linked to produce contamination has increased from 0.7% in the 1970s *(34)* to 13% between 1990 and 2005 *(12).* During this time, new pathogens were identified, diagnostic methods improved, and foodborne disease surveillance systems were enhanced. From 1990 to 2005, as many as 713 outbreaks and approximately 34,000 cases of illness were associated with consumption of contaminated produce *(12).* High-profile outbreaks were linked to cantaloupe contaminated with *Salmonella enterica* serotype Poona, green onions contaminated with hepatitis A virus, lettuce contaminated with *Escherichia coli* O157:H7, raspberries contaminated with *Cyclospora cayetanensis,* and parsley contaminated with *Shigella sonnei (1, 6, 8, 16, 17, 37).* More recently, approximately 200 individuals fell ill during an outbreak of *E. coli* O157:H7 when they consumed fresh spinach *(10),* and multiple outbreaks have been associated with the consumption of Roma tomatoes contaminated with various *Salmonella* serotypes *(9).*

Produce contamination can occur through a number of mechanisms and at various steps during growing, harvesting, packing, and distribution (reviewed in *(25)*). Produce may come into contact with contaminated fertilizers, irrigation water, infected wild or domestic animals, or be han-

dled by infected workers *(18, 26, 30).* Because the occurrence of contamination with microbial pathogens is generally low, we *(21, 22)* and others *(2, 11, 19, 27–29, 31)* have used microbial indicators to study produce quality and safety. We assayed for total aerobic bacteria (aerobic plate count [APC]) and total coliforms as general indicators of produce quality *(11, 21, 22, 29). Enterococcus faecalis* and *Enterococcus faecium* (i.e., "fecal streptococci") have previously been used as indicators of fecal contamination, and our work has shown that approximately 75% of the naturally occurring *Enterococcus* strains isolated from produce belonged to one of these two species *(20).* Therefore, we assayed for total enterococci as an additional indicator of produce quality and a potential indicator of fecal contamination. Because *E. coli* is shed from the intestinal tract of humans and animals, we also assayed for generic *E. coli* as an indicator of fecal contamination *(11, 21, 22)* and the potential presence of enteric pathogens of fecal origin. In packing sheds, we and others have shown that microbial concentrations on certain produce types are significantly higher at end stages compared with early stages of handling *(5, 21).* It is not clear whether this effect is common to all produce items, or for that matter, to all packing sheds. In addition, it would be useful to identify specific steps in the packing process that are associated with higher microbial concentrations, to begin to ascertain the exact mechanisms of produce contamination. Last, there is a need to quantify

the association between concentrations of fecal indicators (e.g., *E. coli*) on produce in farms and packing sheds, to design future strategies aimed at controlling the most likely sources of contamination.

To address these needs, our goal was to identify specific factors associated with elevated microbial concentrations on produce and to quantify their associations with the presence of *E. coli* on produce from farms and packing sheds. We collected 923 produce samples (grown in the southern United States or in northern border states in Mexico) from multiple processing locations in 15 farms and eight sheds in the southern United States. In our previous reports from this study, we used relatively simple statistical techniques, and we did not explore the effects of specific produce types, packing shed steps, or individual packing sheds or farms *(21, 22)*. Here, we take the statistical analyses of the data one step further by (i) reporting on individual factors associated with high concentrations of microbial contamination (univariate analyses), (ii) comparing microbial concentrations at the beginning and end stages of the packing process, and (iii) quantifying the combined effect of the identified factors on produce contamination (multivariate models). The results help identify points along the production and packing chain that may be most appropriate for targeted mitigation strategies to prevent elevated microbial counts and potential fecal contamination.

MATERIALS AND METHODS

Produce sample collection. Samples of 14 types of produce (arugula, broccoli, cabbage, cantaloupe, celery, Swiss chard, cilantro, collards, dill, kale, mustard greens, parsley, spinach, and turnip greens) were collected from 15 farms and eight packing sheds from the southern United States from November 2000 to December 2003. Produce sampling has been described in detail in our two previous reports from this study *(21, 22)*. Samples were collected from fields before and during harvest, and from a number of steps during the packing process. These steps, and therefore sampling locations, varied by produce type. The packing shed steps included the bin in which the produce was brought from the field, the wash tank, the turntable, the rinse cycle, the conveyor belt, and the final packing box. The turntable was used to move leafy greens through the rinse cycle. In U.S. packing sheds, we also sampled produce items grown in Mexican states that border the United States. The majority (147 of 156) of Mexican produce samples were collected from the box immediately after washing and packing. No Mexican produce samples were collected directly from farms in Mexico. For convenience to the reader, we will refer to United States–grown produce (from the southern United States) as "American produce," and Mexican-grown produce (from the northern Mexican border states) as "Mexican produce." This naming convention does not imply that these regionally collected produce samples are representative of all American and Mexican produce.

General microbial quality. Two sets of samples of 400 to 600 g of produce were collected during any one sampling location and/or time for the 14 types of produce. Samples were packed in coolers with ice packs, shipped overnight to North Carolina State University, and immediately analyzed on receipt for indicators (APC, total coliforms, total *Enterococcus,* and total *E. coli*) of microbial quality as previously described *(21, 22)*. Analysis of all samples was initiated within 24 h of sample collection. The limit

of detection of the microbial assays was 10 CFU/g. To avoid under- and overrepresentation of sample counts, when enumerative results fell below the assay limit of detection (10 CFU/g), they were assigned a value halfway between 0 and the detection limit (5 CFU/g or 0.70 log CFU/g) *(14, 32)*.

Statistical analyses. Data analysis was conducted using SAS version 9 (SAS Institute, Inc., Cary, N.C.). APC, total coliforms, total *Enterococcus,* and total *E. coli* concentrations were normalized using log transformation. *E. coli* concentrations were not normally distributed, even after transformation, so they were analyzed using nonparametric methods or by treating *E. coli* concentrations as a binary variable (presence or absence). Comparison of means was done using the Student's *t* test for pairwise comparisons or analysis of variances with Tukey's test for multiple comparisons *(38)*. For *E. coli,* a Mann-Whitney test was performed for pairwise comparisons or a Kruskal-Wallis test with Tukey's test for multiple comparisons. The occurrence of coliforms, *E. coli,* and *Enterococcus* was calculated as the proportion of samples with detectable (greater than the 0.70 log CFU/g) concentrations of the specific microbial indicator relative to total number of samples in that commodity group. Pairwise comparisons of occurrence were done by chi-square test, or Fisher's exact test in the case of small sample sizes. Multiple comparisons were done by a SAS macro using a Tukey-type multiple comparison procedure after an overall chi-square test indicated all comparisons were significant *(13, 38)*. Seasons were defined as fall (September, October, and November), winter (December, January, and February), and spring (March, April, and May). Summer (June, July, and August) was not included because our produce items were not harvested or processed during the summer. A two-sided $P < 0.05$ was considered significant. To reduce the likelihood of confounding by other factors, specific descriptive analyses were restricted to certain subgroups (e.g., samples of American origin only, samples from specific packing steps only).

We used a multivariate, predictive logistic regression model to assess significant predictors of *E. coli* contamination on produce. The output of the logistic model is an odds ratio that estimates the effect of one factor (e.g., season) adjusted for other variables in the model (e.g., the type of produce). *E. coli* concentrations were dichotomized, and values above 0.70 log CFU/g (the value assigned to samples below the limit of detection) were coded as "1," while those equal to 0.70 log CFU/g were coded as "0." To fulfill the assumptions of a logistic model, we only included produce types with samples both above and below the limit of detection for *E. coli* and excluded produce types for which all samples were below the limit of detection for *E. coli*. The category that provided the most stable estimate was used as the referent group for each categorical variable. After screening for collinearity, we developed models that included variables for produce type, origin, packing step, season, and processing location (farm versus shed). A backward selection procedure generated a model that contained only significant predictors of *E. coli* contamination on produce. Stepwise and forward variable selection methods were used to verify the results of the backwards elimination method.

Produce samples obtained from handling steps within the same specific farm or shed may have a similar likelihood of *E. coli* contamination. Therefore, *E. coli* concentrations for samples from the same farm or shed may be correlated, and thus these data could violate the assumption of independence of the logistic regression model. To account for correlations of produce samples within the same farms and sheds, we used a generalized linear mixed model, created using the GLIMMIX macro in SAS. This is a logistic regression model that can account for correlations

TABLE 1. *Indicator concentrations by produce item, including all seasons and processing locations, American produce only*[a]

| Produce item | n | E. coli | | APC[b] | Coliforms | | Enterococcus | |
		Occurrence (%)	Concn (log CFU/g)	Concn (log CFU/g)	Occurrence (%)	Concn (log CFU/g)	Occurrence (%)	Concn (log CFU/g)
Arugula	15	0	0.7 ± 0.00	5.8 ± 0.13^{c}	100	3.4 ± 0.32	$60^{c,d,e}$	$2.1 \pm 0.33^{c,d,e}$
Cabbage	58	29	1.1 ± 0.09	$5.7 \pm 0.08^{c,e,f}$	59^{c}	$1.6 \pm 0.11^{c,e,g}$	98	$3.3 \pm 0.13^{c,e,f}$
Cantaloupe	126	25	1.2 ± 0.10	6.7 ± 0.06	90	3.0 ± 0.11	100	4.1 ± 0.09
Celery	44	2	0.7 ± 0.04^{c}	$4.6 \pm 0.09^{c,d,e,f,g}$	$23^{c,e,f,g}$	$0.9 \pm 0.05^{c,e,f,g}$	$18^{c,d,e,f,g}$	$0.9 \pm 0.07^{c,d,e,f,g}$
Cilantro	187	25	1.1 ± 0.06	6.4 ± 0.07	67	2.1 ± 0.09^{c}	$69^{c,d,e}$	$2.3 \pm 0.10^{c,d,e,g}$
Collards	27	$0^{c,d,f}$	0.7 ± 0.00^{c}	$4.4 \pm 0.17^{c,d,e,f,g}$	$22^{c,e,f,g}$	$1.0 \pm 0.12^{c,e,f,g}$	$37^{c,d,e,g}$	$1.0 \pm 0.10^{c,d,e,f,g}$
Dill	21	$0^{c,d,f}$	0.7 ± 0.00	$5.2 \pm 0.14^{c,e,f,g}$	95	2.4 ± 0.22	95	$3.1 \pm 0.22^{c,e}$
Kale	9	0	0.7 ± 0.00	$4.9 \pm 0.13^{c,e,f,g}$	67	1.3 ± 0.17^{c}	$0^{c,d,e,f,g}$	$0.7 \pm 0.00^{c,d,e,f,g}$
Mustard greens	70	13	1.0 ± 0.11	6.2 ± 0.11^{c}	79	2.4 ± 0.16^{c}	100	4.3 ± 0.15
Parsley	141	11	0.9 ± 0.04^{c}	$6.0 \pm 0.08^{c,f}$	81	2.4 ± 0.10^{c}	89	$3.0 \pm 0.11^{c,e,f}$
Spinach	27	$0^{c,d,f}$	0.7 ± 0.00^{c}	$5.8 \pm 0.16^{c,f}$	63	$1.5 \pm 0.15^{c,g}$	78	$2.1 \pm 0.17^{c,d,e,g}$
Swiss chard	9	0	0.7 ± 0.00	$5.3 \pm 0.21^{c,f}$	$0^{c,d,e,f,g}$	$0.7 \pm 0.00^{c,e,f,g}$	78	$1.6 \pm 0.20^{c,d,e,g}$
Turnip greens	33	$0^{c,d,f}$	0.7 ± 0.00^{c}	5.9 ± 0.13^{c}	61^{c}	$1.5 \pm 0.17^{c,e,g}$	$61^{c,d,e}$	$1.7 \pm 0.18^{c,d,e,g}$
All	767	16	1.0 ± 0.03	6.0 ± 0.04	70	2.2 ± 0.05	78	2.8 ± 0.06

[a] Values are means ± SE. Because 0.70 is the limit of detection (described in "Materials and Methods"), values of 0.70 represent produce with undetectable microbial indicators.

[b] All products had detectable concentrations of APC bacteria (i.e., 100% occurrence).

[c] $P < 0.05$ compared with cantaloupe.

[d] $P < 0.05$ compared with cabbage.

[e] $P < 0.05$ compared with mustard greens.

[f] $P < 0.05$ compared with cilantro.

[g] $P < 0.05$ compared with parsley.

within clusters of samples taken from the same farm or packing shed. We included a random effect for the combination of packing step and farm or shed (e.g., field from farm A or box from shed H) to account for the correlation between samples from the same processing location within a particular farm or shed. The random effect was assumed to have a normal distribution with mean 0 and variance σ^2. If the variance was significantly greater than 0, then the random effect was considered significant and had to be included in the model. A significant random effect suggests that *E. coli* presence among samples within the same handling step at a specific farm or shed are correlated. In other words, a significant random effect suggests that the presence of *E. coli* on produce was significantly affected by that specific step within a specific farm or shed.

RESULTS

To identify the factors associated with the presence of *E. coli,* an indicator of potential fecal contamination, we first investigated whether specific produce types had significantly higher *E. coli* occurrence, as determined by the proportion of samples with concentrations above 0.70 log CFU/g (limit of detection), when compared with other product types. We limited this analysis to the 767 (83%) produce samples grown in the United States. One hundred twenty (16%) American produce samples had detectable concentrations of *E. coli*. Only 6 of the 14 produce types had any detectable *E. coli*: cabbage, cantaloupe, celery, cilantro, mustard greens, and parsley (Table 1). In general, these six produce items also had significantly higher mean concentrations of APC, coliforms, and *Enterococcus* compared with other produce items. The occurrence of detectable concentrations of coliforms and *Enterococcus* ranged

from 0 to 100% and varied significantly by product type ($P < 0.001$). In summary, some specific produce items had significantly higher mean concentrations of microbial indicators than others had.

To determine whether the origin of produce (American or Mexican) affected the concentrations and occurrence of microbial indicators within a given type of product, we compared microbial concentrations between American and Mexican samples of the same produce type (cabbage, cantaloupe, cilantro, and parsley). This analysis was restricted to the 258 (28% of all produce) samples obtained from the box at the end of the washing and packing process, because this was the location that included samples of both U.S. and Mexican origin. In general, we did not find a clear pattern or association between country of origin and any specific microbial concentrations on these produce types (data not shown). In conclusion, the origin of the produce did not seem to be clearly associated with the concentrations or occurrence of specific microbial groups, at least within the confines of our sample set.

We also assessed the relationship between season (fall, winter, and spring) of produce sampling and microbial indicator concentrations (Fig. 1). In order to make comparisons between the three seasons during which samples were obtained (fall, winter, and spring), this analysis was limited to the 382 (41%) samples of American origin that had been sampled in all three seasons. For cilantro and parsley, APC, *E. coli,* coliform, and *Enterococcus* concentrations were significantly higher in fall than they were in spring or winter, and higher in spring than winter. For collards and spin-
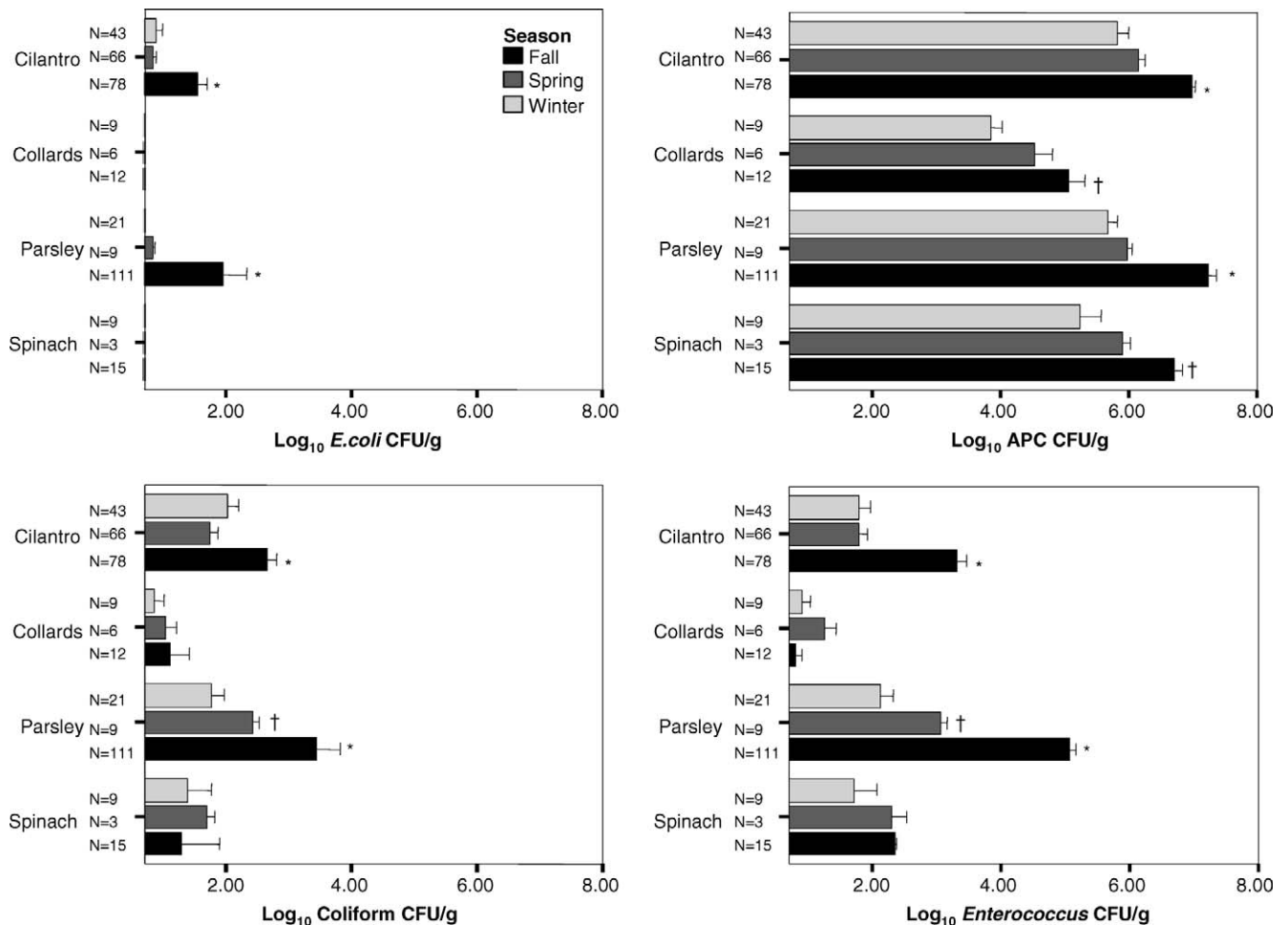
FIGURE 1. *Microbial indicator concentrations for specific types of American produce vary significantly by season. Bars represent mean microbial concentrations for produce items collected during each season. Error bars represent standard error of the mean. Sample sizes are indicated to the left of the bars.* *P < 0.05 compared with Spring and Winter; †P < 0.05 compared with Winter.

ach, we only observed significantly higher APC concentrations in fall compared with winter but not spring. No significant differences in concentrations of the other indicators were observed. In summary, season seems to be associated with microbial quality of produce items.

To determine whether the packing process affected microbial concentrations, we compared the concentrations of the various microbes from samples obtained just prior to packing (in the field) to those obtained at the final stage of packing (in the box) (Fig. 2). The beginning and end stages of packing were the only steps shared by all produce items, and therefore were appropriate choices for this univariate analysis of all produce. This analysis was limited to those 390 (42%) produce samples collected immediately before and after packing, meaning that it was also limited to produce samples of U.S. origin. In general, the majority of produce items had higher microbial concentrations in the final packing box compared with the field. No produce item obtained right after harvest had significantly higher counts of any of the indicator groups when compared with microbial concentrations after packing. Cantaloupe and cilantro samples obtained from the box had significantly higher concentrations of *E. coli* than had field samples. Similar trends were seen for APC (for cabbage, cantaloupe, cilantro, and

parsley), coliforms (for cantaloupe, cilantro, and parsley), and *Enterococcus* (for arugula, cantaloupe, and parsley). In conclusion, the microbial concentrations for most crops were higher, though not always significantly, after the packing process.

Because individual produce types vary in their postharvest handling, in our univariate analysis, we were not able to examine specific steps in the packing process while simultaneously adjusting for the effect of produce type. Therefore, we employed a multivariate regression model to identify significant predictors of the presence of *E. coli*, while simultaneously adjusting for the effect(s) of other predictors, as a function of specific steps during washing and packing, produce types, produce origin, and season (Table 2). This analysis was limited to the 755 (82%) samples from the six produce items that were found in earlier analyses to have detectable concentrations of *E. coli* (occurrence greater than 0%; see also Table 1). Thus, only produce items with at least one sample "positive" for *E. coli* were included in the model, and the remaining produce items (arugula, broccoli, collards, dill, kale, spinach, Swiss chard, and turnip greens) were excluded. An effect for the individual packing shed or farm was not included because data at this level became too sparse. After backwards elim-
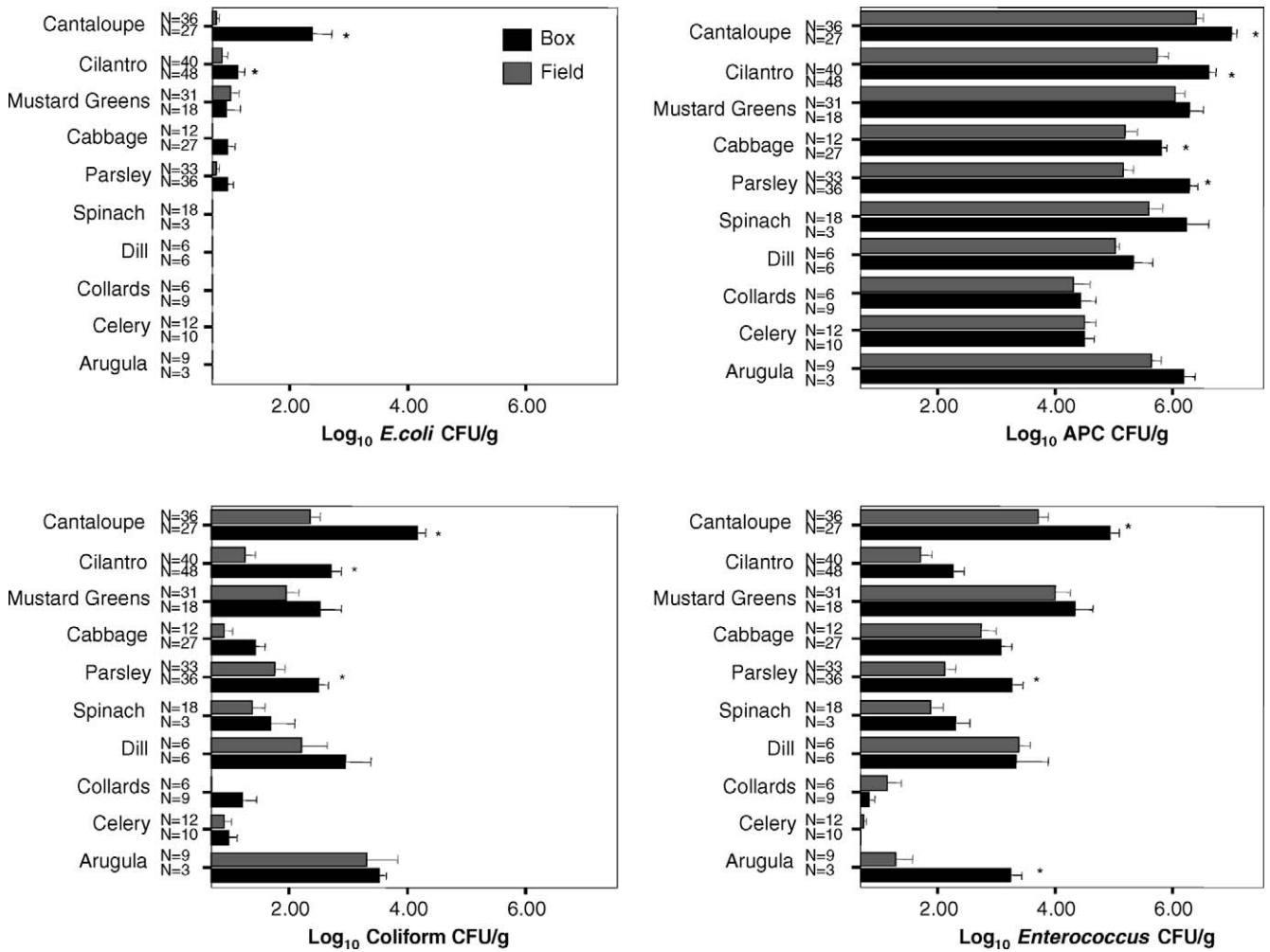
FIGURE 2. *Microbial indicator concentrations for specific types of American produce vary significantly by packing step. Bars represent mean microbial concentrations for produce items collected either from the beginning step (Field) or from the end step (Box). Error bars represent standard error of the mean. Sample sizes are indicated to the left of the bars. *P < 0.05 compared with Field.*

ination, the final model included all variables significantly associated with the presence of *E. coli* and contained variables representing produce type, packing step, origin of sample, and season. After controlling for all other variables in the model, cabbage was approximately three times more likely, and cantaloupe approximately four times more likely to be positive for *E. coli* when compared with parsley (Table 2, "Logistic regression model," and Fig. 3). Relative to field samples, those produce items obtained from the packing bins had over a sixfold increase in likelihood of *E. coli* contamination, while this likelihood was fourfold and threefold increased for samples that originated from the box or conveyor belt, or from the turntable, respectively. Produce samples grown in the United States were almost eight times more likely to have detectable *E. coli* compared with those grown in Mexico. Produce sampled in the fall was over six times as likely to be contaminated with *E. coli* as produce sampled in the winter (Table 2, "Logistic regression model").

To account for the potential lack of independence between samples obtained from the same step within the same farm or shed, a random effects term for the packing step within a specific farm or shed was included in the model (Table 2, "GLMMIX logistic regression model"). The random effect was found to be significant (data not shown), suggesting that the microbial quality of produce was significantly affected by the particular step in the packing process within a specific shed or farm, even after adjusting for type of produce, origin, and season. Similar predictors were found to be significant in both models; however, the magnitudes of these associations were slightly greater in the GLMMIX model compared with the ordinary logistic regression model. In conclusion, the type of produce, packing step, origin, season, and specific shed or farm were all significantly associated with the likelihood of contamination with *E. coli.*

DISCUSSION

Our goal for the present study was to identify specific factors associated with elevated concentrations of microbiological contamination on produce, and to quantify their association with *E. coli* contamination on produce sampled from farms and packing sheds. From our univariate analyses, we found that produce type, washing and packing steps, and season were associated with elevated APC, coliforms, *E. coli,* and *Enterococcus* counts. The multivariate

TABLE 2. *Logistic regression models predicting the presence of* E. coli *on produce samples* (n = 755)[a]

Parameter	Logistic regression model					GLMMIX logistic regression model				
	Beta Coefficients	SE	OR	95% CL		Beta Coefficients	SE	OR	95% CL	
Intercept	−5.756	0.688				−4.880	0.714			
Location										
Bin[b]	1.913	0.437	6.77	2.87	15.96					
Box[b]	1.434	0.362	4.20	2.06	8.53					
Conveyor belt[b]	1.394	0.463	4.03	1.63	9.99					
Turntable[b]	1.155	0.553	3.17	1.07	9.38					
Rinse cycle	0.737	0.454	2.09	0.86	5.08					
Wash tank	0.758	0.495	2.13	0.81	5.63					
Field				Referent						
Produce item										
Cabbage[b]	1.074	0.403	2.93	1.33	6.45	1.614	0.615	5.02	1.50	16.76
Cantaloupe[b]	1.293	0.402	3.65	1.66	8.01	1.348	0.401	3.85	1.76	8.44
Celery	−1.255	1.093	0.29	0.03	2.43	−1.335	1.150	0.26	0.03	2.51
Cilantro	0.622	0.343	1.86	0.95	3.65	0.513	0.360	1.67	0.82	3.38
Mustard greens	0.798	0.485	2.22	0.86	5.75	0.577	0.399	1.78	0.81	3.89
Parsley				Referent					Referent	
Origin										
American[b]	2.062	0.404	7.87	3.56	17.37	2.240	0.574	9.39	3.05	28.92
Mexican				Referent					Referent	
Season										
Fall[b]	1.859	0.392	6.42	2.98	13.83	2.003	0.512	7.41	2.72	20.21
Spring	0.305	0.356	1.36	0.68	2.73	0.146	0.454	1.16	0.47	2.82
Winter				Referent					Referent	
Processing location within specific farm/shed						0.680	0.284			
Residual variation						0.949[c]	0.050			

[a] SE, standard error; OR, odds ratio; CL, confidence limit
[b] Indicates significant OR.
[c] Value is residual variation.

analyses indicated that produce type, washing and packing steps, season, and origin were significantly associated with the presence of *E. coli* contamination. Our results agree with our previous studies and those of others who found that the microbial concentration associated with certain produce items is affected by handling in sheds *(3, 15, 21, 22, 33)*.

Both our univariate and multivariate analyses highlighted differences in microbial concentration between different produce items. Cabbage, cantaloupe, celery, cilantro, mustard greens, and parsley all had detectable concentrations of *E. coli* and significantly higher number of the other microbiological indicators than had other crops (Table 1). While a 1- to 2-log difference in APC or total coliforms concentrations by produce type is unlikely to have a substantial public health implication, differences in the concentrations of *E. coli* should be viewed with caution, as higher concentrations of this organism may indicate an increased likelihood of fecal contamination. Disease outbreaks, such as those associated with cantaloupe contaminated with *Salmonella (4, 5, 8)* and parsley contaminated with *Shigella sonnei (6)* have occurred. While the effects

of microbial contamination on some produce items (such as mustard greens and cabbage) may be mitigated by cooking, cross-contamination from these products to other foods may still occur. Furthermore, outbreaks associated with mustard greens and cabbage contaminated with foodborne pathogens have also been reported *(7)*. The higher microbial concentrations on these produce items could be due to their physical characteristics, growth conditions, preharvest handling practices, and/or postharvest packing and handling conditions *(3, 4)*. Interestingly, certain produce items (e.g., arugula, collards, dill, Swiss chard, kale, spinach, turnip greens) had no detectable concentrations of *E. coli,* although it should be noted that our sample sizes for these produce items were generally smaller (range of 9 to 33) than for those produce items with detectable *E. coli.*

The majority of produce categories showed higher microbial concentrations, albeit not always significantly higher, when samples were obtained from the box (postpacking) as compared with those collected immediately postharvest. Postharvest increases in microbial concentrations on produce may occur because of contact with contaminated human hands, rinse water (e.g., water without sanitizers or
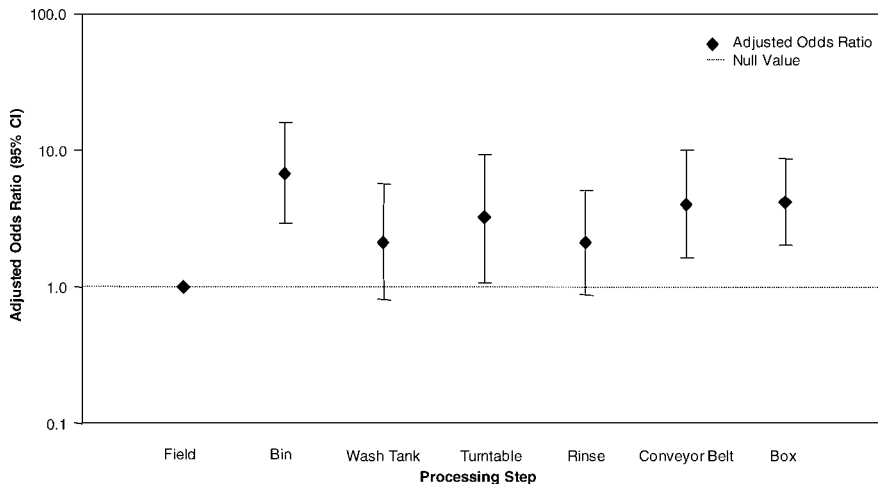
FIGURE 3. *The probability of* E. coli *contamination, adjusted for other factors in the model, varies by packing step. The adjusted odds ratios (diamond) and 95% confidence intervals (error bars) are shown for each of the packing steps. The null value (odds ratio = 1) is shown as a line. Packing steps that have 95% confidence intervals that cross this line have nonsignificant adjusted odds ratios. Because Field is the referent category, the odds ratio is set at 1, and Field has no 95% confidence intervals.*

with inactivated sanitizers), equipment surfaces, animals or their waste products, or other contaminated produce (cross-contamination) (reviewed in (24)). Growth of some microorganisms throughout the farm-packing continuum may also account for this observation and would be important to study in future research. From surveys of farms and sheds, and interviews with farm and shed managers, we found that animals were frequently observed in and near several of the fields and sheds surveyed in this study (data not shown), suggesting that there is potential for fecal contamination of produce originating from domestic animals. Subsequent reports will address the role of shed water and shed equipment surfaces on produce contamination. Taken together, these findings suggest the need for additional postharvest interventions to reduce the likelihood of produce contamination, including improvements in worker hygiene, confirmation of chlorine residual in wash water and/or ice, disinfection of equipment surfaces, and improved biosecurity. Even though sheds offer manageable ways of cleaning and packing produce under controlled conditions, the concept of field packing is worth revisiting for specific products (e.g., cantaloupe) that showed particularly significant increases in microbial contamination during postharvest handling.

We observed seasonal differences in microbial concentrations on produce. This observation may have been an artifact caused by oversampling of produce items with higher microbial concentrations in a particular season (e.g., fall). However, Figure 1 shows that this effect is present even when the data are stratified by produce item. Furthermore, even after adjusting for the effects of other variables, we observed significant seasonal differences in the concentrations of microbial contaminants in our multivariate regression models (Table 2). Therefore, these results suggest that the effect of season is independent of other variables in the model. Season may be a marker for a number of other factors, including climatologic and ecologic changes, changes in rodent and pest populations that come into contact with the produce, and changes in human behavior. Because our produce types were not harvested or packed during the summer, we could not assess the impact of a summer season in our study. Additionally, the effect of season may be par-

ticular to the geographic regions in the southern United States and northern Mexican border states from which produce originated. Further research should address this factor specifically, to delineate whether the association between season and the concentrations of microbes on produce show similar trends over time and are reproducible in other geographic areas or simply a unique observation associated with our data.

From our multivariate models, we found that American produce was significantly more likely than Mexican produce to have some degree of *E. coli* contamination. Interestingly, previous studies have shown that imported produce was more often contaminated with pathogens than produce of domestic origin (35, 36). In this regard, our findings may be counterintuitive but should be viewed with caution, as the presence of *E. coli* is only suggestive of potential pathogen contamination, and our survey was limited to specific regions in the United States and Mexico and a specific period. Further, we did not collect Mexican samples for all crops, and we may not have collected sufficient numbers of ''high-risk'' Mexican produce (i.e., those most often implicated in disease outbreaks). The microbiological differences in the quality and safety of produce items grown outside the United States relative to items grown in the United States merits additional study.

We found that *E. coli* contamination of produce was associated with specific postharvest packing steps, and with specific farms and sheds, as indicated by the significant random effects in the model (Table 2). One farm or shed might be associated with a greater likelihood of *E. coli* contamination on produce than might another because of a variety of factors, including increased exposure to animals (e.g., biosecurity), poor quality runoff or irrigation water, or the hygiene and sanitation practices particular to that farm or shed. We found that produce samples taken from the bin, box, turntable, and conveyor belt packing shed steps had significantly greater likelihood of *E. coli* contamination than had those taken from the field. All these locations involve direct contact between produce items and equipment surfaces and/or workers' hands. Interestingly, locations in which produce was in contact with water were not significantly associated with *E. coli* contamination. All

sheds used chlorine in their water, and we detected low concentrations or no fecal coliforms or *E. coli* in the water used for produce washing and packing (data not shown). In future reports, we will discuss the association between microbial concentrations on produce and those in water and on equipment surfaces. Further research should address the relationship between workers' hands and produce contamination.

One potential limitation of our study was the use of microbial indicators as a proxy for foodborne pathogens. The public health significance of high APC, coliforms, and *Enterococcus* counts on produce is not clear, and we recognize that these microbial populations are not necessarily indicators relevant to food safety. Some coliforms (e.g., *Klebsiella* spp.) are commonly associated with produce and can multiply under favorable environmental conditions *(23)*. In addition, no research group has yet examined the direct link between *E. coli* and foodborne pathogens on produce. These same produce samples were also screened for the presence of selected foodborne pathogens that were rarely detected *(21, 22)*. This brings up a common problem in studies focusing on naturally contaminated produce, for which the occurrence of pathogen contamination is low. Nonetheless, the frequent detection of *E. coli* contamination on fresh produce is disconcerting, and it deserves further investigation to establish if there are indeed associations between elevated *E. coli* concentrations and the occurrence of foodborne enteric pathogens on fresh produce items.

The multivariate analytical approach of examining multiple produce items and various factors in produce production from farm to packing shed is novel and well suited to examining this complex process. With this approach, we were able to determine the magnitudes of association between individual farming and shed factors and produce contamination. This information is valuable for informing future mitigation strategies (reviewed in *(24)*). For example, based on our findings, specific interventions for individual produce types (e.g., cantaloupe, cabbage) may be developed, and certain steps (e.g., placing produce on conveyor belts) in the packing process may be considered as critical control points. This approach also will facilitate identification of the mechanism(s) by which produce items become contaminated with microbes, or by which microbial concentrations increase, as well as providing a means by which to measure the impact of specific interventions in farms and sheds.

In conclusion, contamination of produce with *E. coli* is affected by the type, origin, season of harvest, postharvest packing process steps, and by the specific farm or shed. Since 2000 to 2003, when our data was collected, the produce industry has implemented a number of important improvements, and there is increased attention to "good agricultural practices" that theoretically should reduce the likelihood of pathogen contamination of fresh produce. Our data may serve as a baseline measure to compare the impact of these improvements as well as a guide for examining potential mechanisms of contamination. These data are also useful for past and future comparisons to similar studies of produce handling, which may yield variable results due to the complex ecosystems studied. Reproducible findings and common themes from this and other studies may also be useful in informing risk models and in the consideration of potential interventions to control microbiological contamination of fresh produce.

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