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Abstract

BACKGROUND: Farmers' markets have been growing in popularity in the United States, but the microbial quality and safety of the food sold at these markets is currently unknown. The purpose of this study was to assess the microbial safety and quality of fresh basil, parsley, and cilantro sold at farmers' markets in the Los Angeles, Orange County, and greater Seattle areas.

RESULTS: A total of 133 samples (52 basil, 41 cilantro, and 40 parsley) were collected from 13 different farmers' markets and tested for *Salmonella* and generic *Escherichia coli*. One sample (parsley) was confirmed positive for *Salmonella* and 24.1% of the samples were positive for generic *E. coli*, with a range of 0.70-3.15 log CFU/g. Among the herbs tested, basil showed the highest percentage of samples with generic *E. coli* (26.9%), followed by cilantro (24.4%), and then parsley (20.0%). For 12% of samples, the levels of generic *E. coli* exceeded guidelines established by the Public Health Laboratory Service for microbiological quality of ready-to-eat foods.

CONCLUSION: Overall, this study indicates the presence of *Salmonella* and generic *E. coli* in fresh herbs sold at farmers' markets; however, additional studies are needed to determine the sources and extent of contamination.

Introduction

Farmers' markets have become an important source of produce for many consumers in the United States.[1] These markets are generally held in the summer months and allow for consumers to purchase locally grown fruits and vegetables directly from the producer or farmer. According to the United States Department of Agriculture (USDA) Economic Research Service, farmers' markets have been increasing since 2009 near urban areas, particularly along the East and West coasts.[1] In August 2013 there were over 8,000 farmers' markets listed in the USDA's National Farmers' Market directory, a 3.6% increase from 2012.[2] While farmers' markets can become certified to ensure that each farmer is actually growing the commodities being sold, food safety is not addressed as part of the certification process. Some potential areas of concern with regard to food safety at these markets are the storage conditions of the produce throughout the day, the farming practices, and the farmer's pre- and post- harvest handling techniques.

Certain herbs, such as parsley, basil, and cilantro have been implicated in many food outbreaks over the past two decades.[3-6] In 1999, there were 41 restaurant-associated illnesses and 35 sporadic cases involving *Salmonella enterica* serotype Thompson in fresh, room temperature cilantro that was suspected to originate from Mexico.[5] In 2006, about 200 teachers and students in Denmark were infected with *Salmonella* and enterotoxigenic *Escherichia coli* (ETEC) from the consumption of a pasta salad with pesto.[3] A retrospective cohort study determined that fresh basil used in the preparation of the pesto was most likely the source of illness due to contamination with ETEC and *S. enterica* serotype Anatum.[3] Furthermore, in 2007, *Salmonella* was found in 18 out of 3,760 ready-to-eat fresh herb samples collected from different retail stores and tested by 30 laboratories in the UK.[6] Eight of the 18 contaminated samples consisted of fresh basil obtained from a single grower in Israel.[4,6] Increasing concern

69 over foodborne outbreaks in fresh produce has also led to testing for generic *E. coli* as an
70 indicator of fecal contamination and potential pathogen presence.[7] Fecal contamination in fresh
71 herbs and other types of fresh produce is problematic, as these items are commonly consumed
72 raw, with no intervention step to inactivate potential pathogens.

73 There is currently limited information on food safety at farmers' markets and some
74 studies conducted thus far have reported concerning results.[8-10] For example, a study in
75 Pennsylvania, USA, reported the presence of *Salmonella* and *Campylobacter* in raw chicken
76 sold at farmers' markets at detection frequencies of 28% and 90%, respectively.[9] In
77 comparison, raw chicken samples from conventional supermarkets showed detection
78 frequencies of 8-20% for *Salmonella* and 28-52% for *Campylobacter*. Teng *et al.*[10] investigated
79 the food handling practices of cheese vendors at farmers' markets located in Ontario, Canada. It
80 was found that 47% of the vendors had problems with refrigeration and a majority of the
81 vendors did not wash their hands prior to handling the cheese. Furthermore, a study surveying
82 supermarkets and farmers' markets in Ontario, Canada, reported the presence of thermotolerant
83 *Campylobacter* spp. in a number of fresh produce items sold at the farmers' markets, including
84 parsley, and no detections in fresh produce items sold at the supermarkets.[8] Despite the
85 potential for foodborne illness from fresh herbs and other fresh produce sold at farmers' markets
86 in the United States, there is currently a lack of knowledge regarding the microbial safety and
87 quality of these items.

88 Due to the prevalence of farmers' markets along the U.S. West Coast and the association
89 of fresh herbs with outbreaks of foodborne illness, the overall objective of this study was to
90 conduct a survey of the microbial safety and quality of fresh basil, parsley, and cilantro sold at

91 farmers' markets in the Los Angeles, Orange County and greater Seattle areas. Specifically, the
92 fresh herbs were tested for *Salmonella, E. coli,* and total coliforms.

93 **Materials and Methods**

94 **Media and bacterial strains**. Unless otherwise stated, all media were obtained from Hardy
95 Diagnostics (Santa Maria, CA, USA). For the Los Angeles County, CA, and Orange County,
96 CA, portion of the study, *S. enterica* serotype Abaetetuba ATCC 35640 and generic *E. coli*
97 ATCC 51813 were used as positive control strains. For the greater Seattle area, WA, portion of
98 the study an environmental *S. enterica* isolate, *S. enterica* LT2 (courtesy of the laboratory of Dr.
99 Sobsey at the University of North Carolina, Chapel Hill, USA), and generic *E. coli* ATCC
100 11303 were used as positive controls.

101 **Sample collection.** Thirteen different farmers' markets were visited in the Los Angeles,
102 Orange County and greater Seattle areas (Table 1), and a total of 133 samples of basil, parsley,
103 and cilantro were aseptically collected from the display tables using plastic sampling bags.
104 Farmers' markets were selected on the basis of geographical proximity to the research
105 laboratories to allow for samples to be analyzed on the same day that they were collected. The
106 number of samples collected was determined based on budgetary constraints as well as the
107 availability of samples at farmers' markets. Sample collection took place between 8 and 10 am
108 in Orange and Los Angeles Counties and between 10:30 am and 2:30 pm in the greater Seattle
109 area. Samples were collected between July and October 2013 and each farmers' market was
110 visited between 1 and 3 times depending on sample availability (Table 1). Each sample unit
111 collected was equivalent to at least 454 grams (1 pound).[11] Following sample collection, herbs
112 were transported on ice in a cooler to the laboratory at Chapman University (Orange, CA, USA)

113 or the University of Washington (Seattle, WA, USA), where they were prepared according to
114 the methods described in succeeding sections.
115 ***Salmonella* testing of fresh herbs.** Samples were prepared for *Salmonella* testing according to
116 the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM).[12]
117 Twenty-five grams of each herb sample were aseptically weighed into 24 oz. Whirl-Pak bags
118 (Nasco, Fort Atkinson, WI, USA). Lactose broth (225 ml) was added and mixed by vigorously
119 swirling the bag 25 times clockwise and then counterclockwise. The samples were incubated for
120 24 ± 2 h at 35 ± 2 ºC. Then, 0.1 ml of each sample was transferred to a test tube containing 10
121 ml of Rappaport Vassiliadis (RV) broth and 1.0 ml of each sample was transferred to a test tube
122 containing 10 ml tetrathionate (TT) broth. The inoculated RV and TT tubes were incubated for
123 24 ± 2 h at 42 ± 1 ºC. Next, a sterile inoculating loop was used to streak samples from the RV
124 and TT tubes onto individual plates of xylose lysine deoxycholate (XLD), bismuth sulfite (BS),
125 and hektoen enteric (HE) agar for isolation, resulting in six plates per sample. The plates were
126 inverted and incubated for 24 ± 2 h at 35 ± 2 ºC.
127 After incubation, typical *Salmonella* colonies were selected from XLD, BS, and HE agar
128 plates and confirmed, as described below. Typical colonies on XLD agar appear pink with or
129 without black centers.[12] Typical colonies on BS agar appear brown, gray or black with an
130 occasional metallic sheen, and typical colonies on HE agar appear blue to blue-green with or
131 without black centers. In the absence of typical colonies on HE and XLD after 24 ± 2 h
132 incubation, one atypical *Salmonella* colony was selected per sample for confirmation testing. If
133 typical or suspicious colonies were not present on BS agar after 24 ± 2 h, the plates were re-
134 incubated for an additional 24 ± 2 h. If typical or suspicious colonies were not present after 48
135 ± 2 h incubation, then one atypical colony was selected per sample for confirmation testing.[12]

136 The colonies were transferred to triple sugar iron (TSI) agar and lysine iron agar (LIA) slants
137 and incubated at 35 ± 2°C for 24 ± 2 h. Samples showing typical TSI/LIA slants were then
138 confirmed with API 20E test kits (bioMérieux, Durham, NC, USA).
139 **Generic *Escherichia coli* and total coliform testing of fresh herbs**. Herbs were tested for
140 generic *E. coli* and total coliforms according to the Association of Official Analytical Chemists
141 Method 991.14.[13] Samples (50 g each) were aseptically weighed into Whirl-Pak bags and 450
142 ml of Butterfield's phosphate buffer was added. Samples were then mixed at 230 rpm for 30 s
143 in a Stomacher 400 Circulator (Seward, Norfolk, UK). Each sample was plated in duplicate by
144 pipetting 1 ml of the sample homogenate onto an *E. coli*/Coliform Petrifilm plate (3M, Saint
145 Paul, MN, USA). The Petrifilm plates were incubated at 35 ± 2 ºC for 48 ± 2 h in stacks of 20
146 or less and then enumerated for *E. coli* and total coliforms. The average *E. coli* and total
147 coliform counts were determined for each sample. In cases where the number of colonies was
148 outside of the countable range of 15-150, an estimated plate count was obtained.
149 **Statistical analyses.** The levels of *E. coli* and total coliforms were statistically compared across
150 herb types using a one-way analysis of variance (ANOVA), with a predetermined significance
151 level of $p < 0.05$. The percentages of samples that were positive for *E. coli* and total coliforms
152 were compared across herb types with a Pearson's chi-square test, with a pre-determined 2-
153 sided significance value of $p < 0.05$. All statistical analyses were carried out using IBM SPSS
154 Statistics 21 (IBM SPSS Inc., Armonk, NY, USA).

155 **Results and Discussion**

156 **Sample collection.** Overall, 133 samples of fresh herbs were collected for testing from 13
157 different farmers' markets (Table 1). Samples were collected from 49 different vendors at these
158 markets, with an average of 3 samples collected per vendor. Among the samples collected,

basil represented the highest percentage (39%), followed by parsley (30%), and then cilantro (31%). Figure 1 provides a breakdown of the number of each type of herb collected within the three major geographic sampling regions of Orange County, Los Angeles, and the greater Seattle areas. The greatest number of samples was collected in Orange County, CA (n = 68), followed by the greater Seattle area, WA (n = 41), and Los Angeles County, CA (n = 24).

***Salmonella* results.** Of the 133 samples collected, 15 samples had typical or suspicious growth on HE, XLD, and/or BS agar. However, only one sample confirmed positive for *Salmonella* on TSI/LIA and the API 20E test strip. This was a sample of parsley collected from a Los Angeles County farmers' market (LA1) that showed typical growth on both HE and XLD agars. According to the biochemical reactions, the profile given on the API 20E test strip was 6704752 with 99.9% identification of *Salmonella* spp. The remaining 118 samples either showed no growth or atypical colonies on HE, XLD, and BS agars. These samples were ruled out as negative with the TSI/LIA slants and, when necessary, an API 20E test strip.

The overall prevalence of *Salmonella* in parsley was 2.5%. The prevalence of *Salmonella* in fresh herbs found in this study was similar to percentages reported previously for *Salmonella* in FDA field investigation studies.[11,14] These studies reported *Salmonella* prevalence rates of 0-2.5% in imported and domestic parsley samples and 1.2-9% in imported and domestic cilantro samples. The FDA studies each collected 84-90 samples of parsley and 85-177 samples of cilantro, compared to 40 parsley samples and 41 cilantro samples collected in the current study. Further testing of these herbs from farmers' markets will be useful in verifying *Salmonella* prevalence. Although it is not known whether the *Salmonella* detected was present at infectious levels, contamination of fresh herbs with *Salmonella* is concerning considering that these herbs are commonly consumed raw. Salmonellosis symptoms include:

182 diarrhea, abdominal cramps, and fever about 12 to 72 hours after consumption that lasts about
183 four to seven days.[15] In severe cases, the diarrhea may be so detrimental that the patients must
184 be hospitalized because the infection can spread from the intestines to the blood stream and
185 other sites in the body. The severe illness generally occurs in the elderly, infants and those with
186 compromised immune systems. Overall, the results of the current study illustrate the possibility
187 of *Salmonella* contamination in fresh herbs sold at farmers' markets and demonstrate a need for
188 more extensive investigation into this topic.
189 **Generic *Escherichia coli* and total coliform results**. Among the 133 fresh herb samples
190 tested in this study, 24.1% were positive for generic *E. coli* (Fig. 1) and 84.2% were positive for
191 total coliforms, with a range of 0.70-3.15 and 0.70-4.15 log CFU/g, respectively (Table 2).
192 Interestingly, the parsley sample found to be positive for *Salmonella* was positive for total
193 coliform growth (0.70 log CFU/g) but not for *E. coli*. The average generic *E. coli* count for all
194 positive samples combined was 1.81 log CFU/g and the average total coliform count was 2.45
195 log CFU/g. There were no significant differences in levels of *E. coli* or total coliforms when
196 compared across the three types of herbs tested, according to a one-way ANOVA, with
197 significance set at $p < 0.05$. A total of 16 samples had average *E. coli* counts considered to be
198 unsatisfactory (≥ 2 log CFU/g) according to guidelines established by the Public Health
199 Laboratory Service for microbiological quality of ready-to-eat foods.[16] The herbs in this
200 category included seven basil samples, five cilantro samples, and four parsley samples. These
201 samples were collected from two farmers' markets in Orange County, CA (OC1 and OC2), two
202 farmers' markets in the greater Seattle area (SC1 and KC3), and one farmers' market in Los
203 Angeles County, CA (LA2). Among the herbs tested, basil showed the highest percentage of
204 samples with growth for generic *E. coli* (26.9%), followed by cilantro (24.4%) and then parsley

(20.0%). On the other hand, cilantro showed the greatest percentage of samples positive for total coliforms (87.8%), followed by basil (82.7%), and parsley (82.5%). There were no significant differences in the percentage of samples positive for *E. coli* or total coliforms across herb types, according to a Pearson's chi-square test with significance set at $p < 0.05$. As shown in Fig. 1, Orange County farmers' markets had the highest percentage of samples with *E. coli* growth, at 26.5%, followed by farmers' markets in the greater Seattle area (24.4%), and Los Angeles County farmers' markets (16.7%). The percentages of positive samples were not statistically compared across locations due to differences in sample sizes.

Although generic *E. coli* are generally more useful than total coliforms as indicators of fecal contamination in fresh produce, total coliform levels were also recorded in this study in order to enable comparison with existing research on microbiological quality of fresh herbs. In general, the levels and detection frequencies of generic *E. coli* and total coliforms in the current study were similar to or higher than those found in previous studies examining the microbiological quality of fresh herbs. For example, in a series of two studies, Johnston *et al.*[17,18] reported average levels of generic *E. coli* to be 0.70-1.31 log CFU/g and total coliform levels to be 1.3-3.4 log CFU/g for commercially grown parsley (n = 141) and cilantro samples (n = 187) collected during multiple steps in the production and packaging process. In comparison, average levels of 1.82 log CFU/g (generic *E. coli*) and 2.36 log CFU/g (total coliforms) were found in the current study for these two herbs combined (excluding basil). Furthermore, Allen *et al.*[19] tested a variety of herb samples (n = 61), including basil and cilantro, sold at retail stores in five Canadian cities and found that only 6.6% of the samples showed *E. coli* growth and 37.7% of the samples showed growth of total coliforms, compared to 24.1% and 84.2%, respectively, in the current study. However, the average total coliform

228 counts of 1.3 to 2.6 log CFU/g reported by Allen *et al.*[19] were similar to those observed in the
229 current study for all herbs combined (2.45 log CFU/g). Finally, a study by Arthur *et al.*[20] also
230 found lower detection frequencies of generic *E. coli* in fresh herbs sold at retail distribution
231 centers and farmers' markets in Ontario, Canada, with growth in 13.4% of parsley samples (n =
232 127) and 4.9% of cilantro samples (n = 61). Interestingly, the authors reported *E. coli* to be
233 present at higher maximum levels in these herbs as compared to the current study, with up to
234 4.2 log CFU/g found in parsley and up to 3.9 log CFU/g found in cilantro, compared to up to
235 3.15 log CFU/g in parsley and up to 2.66 log CFU/g in cilantro found in the current study
236 (Table 2).

237 Overall, the majority of fresh herb samples tested in the present study were compliant
238 with microbiological criteria established by the Public Health Laboratory Service for
239 microbiological quality of ready-to-eat foods; however, 12% of samples showed levels of
240 generic *E. coli* determined to be unsatisfactory by these guidelines. Further research is needed
241 to determine the source(s) of contamination and whether contamination is greater at farmers'
242 markets compared to other retail sources of fresh produce.

243 **Conclusions**

244 With the growing popularity of farmers' markets, the lack of food safety regulations at
245 these markets, and the association of fresh produce with foodborne illness, it has become
246 increasingly important to monitor the microbiological safety and quality of these items.
247 Overall, a relatively high level of microbiological contamination was found in the herbs
248 collected in this study as compared to previous studies. However, additional studies are needed
249 to verify this trend. While a direct comparison between fresh herbs from farmers' markets and
250 conventional supermarkets was not carried out in this study, storing herbs at ambient

temperatures in the open environment during warm summer days could impact the microbiological safety and quality of these items. At conventional supermarkets, fresh herbs and other perishable produce items are held under controlled temperature and humidity conditions and they are required to be handled according to the Good Manufacturing Practices. In order to assess the importance of these factors, additional research is needed comparing the microbial quality and safety of herbs held in controlled environments, such as those in a conventional supermarket, to those held at ambient temperatures in outdoor environments, such as at a farmers' market. Since farmers' markets are generally held in the summer months, another important area of research will be to monitor microbial changes that occur in fresh herbs and other perishables throughout the day as the temperature increases from the morning to the afternoon. The current study, along with future research in this area, will be important in heightening our understanding of the safety of perishable foods sold at farmers' markets.

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Figure Captions

Figure 1. Number of samples collected from farmers' markets categorized by geographic region and by herb type. The number of samples that tested positive for *Escherichia coli* growth is displayed within the total sample number for each category. The greater Seattle area includes data from both King and Snohomish Counties.

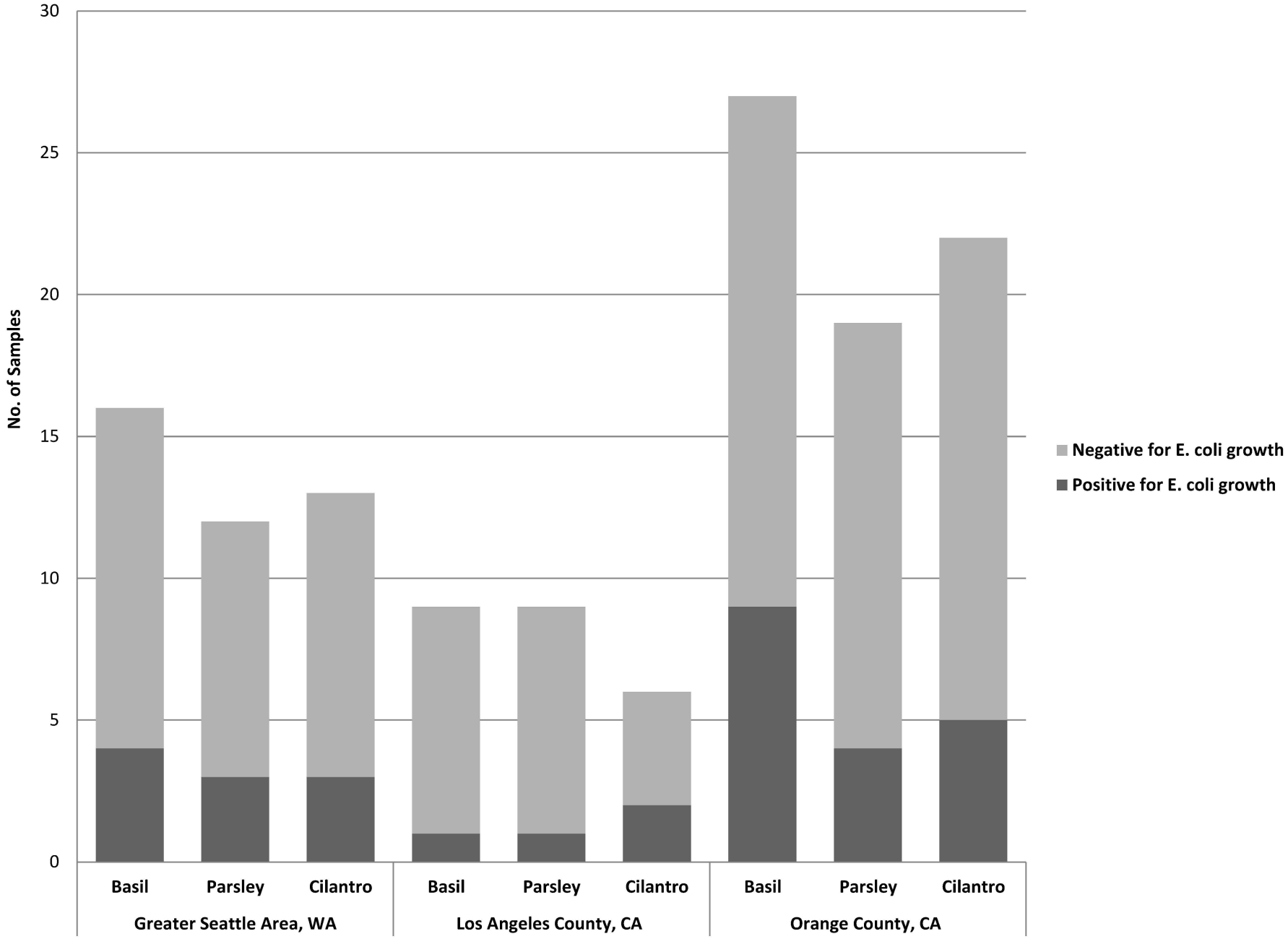
Tables

Table 1. Details on farmers' markets sampled in this study

Location	Farmer's market ID	No. of vendors collected from	No. of times visited	No. of samples collected	Months visited
King County, WA	KC1	1	1	3	August
King County, WA	KC2	1	1	3	August
King County, WA	KC3	7	3	18	September, October
King County, WA	KC4	3	3	7	August, September
Snohomish County, WA	SC1	5	2	10	August, September
Los Angeles County, CA	LA1	4	1	5	August
Los Angeles County, CA	LA2	7	1	16	August
Los Angeles County, CA	LA3	2	1	3	August
Orange County, CA	OC1	13	3	53	July, August
Orange County, CA	OC2	2	2	8	July, August
Orange County, CA	OC3	2	1	3	August
Orange County, CA	OC4	1	1	2	August
Orange County, CA	OC5	1	1	2	August

Table 2. Generic *E. coli* and total coliform levels in positive samples of basil, parsley and cilantro

Herb type	**Generic *E. coli***			**Total coliforms**		
	Positive samples (n)	**Average** (log CFU/g)	**Range** (log CFU/g)	**Positive samples (n)**	**Average** (log CFU/g)	**Range** (log CFU/g)
Basil	14	1.79	0.70-2.95	43	2.61	0.70-4.15
Cilantro	10	1.71	0.70-2.66	36	2.30	0.70-4.08
Parsley	8	1.96	1.00-3.15	33	2.42	0.70-3.75
Overall	32	1.81	0.70-3.15	112	2.45	0.70-4.15


30
25
20
15
10
5
0
No. of Samples
Basil
Parsley
Cilantro
Basil
Parsley
Cilantro
Basil
Parsley
Cilantro
Greater Seattle Area, WA
Los Angeles County, CA
Orange County, CA
Negative for E. coli growth
Positive for E. coli growth