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Longitudinal Microbiological Survey of Fresh Produce Grown by Farmers in the Upper Midwest

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ABSTRACT

Microbiological analyses of fruits and vegetables produced by farms in Minnesota and Wisconsin were conducted to determine coliform and *Escherichia coli* counts and the prevalence of *E. coli, Salmonella,* and *E. coli* O157:H7. During the 2003 and 2004 harvest seasons, 14 organic farms (certified by accredited organic agencies), 30 semiorganic farms (used organic practices but not certified), and 19 conventional farms were sampled to analyze 2,029 preharvest produce samples (473 organic, 911 semiorganic, and 645 conventional). Produce varieties included mainly lettuces, leafy greens, cabbages, broccoli, peppers, tomatoes, zucchini, summer squash, cucumber, and berries. Semiorganic and organic farms provided the majority of leafy greens and lettuces. Produce samples from the three farm types had average coliform counts of 1.5 to 2.4 log most probable number per g. Conventional produce had either significantly lower or similar coliform populations compared with the semiorganic and organic produce. None of the produce samples collected during the 2 years of this study were contaminated with *Salmonella* or *E. coli* O157:H7. *E. coli* contamination was detected in 8% of the samples, and leafy greens, lettuces, and cabbages had significantly higher *E. coli* prevalence than did all the other produce types in both years for the three farm types. The prevalence of *E. coli* contamination by produce type was not significantly different between the three farm types during these 2 years, with the exception of organic leafy greens, in which *E. coli* prevalence was one-third that of semiorganic leafy greens in 2003. These results indicate that the preharvest microbiological quality of produce from the three types of farms was very similar during these two seasons and that produce type appears to be more likely than farm type to influence *E. coli* contamination.

Fresh fruits and vegetables, including fruit juices, are essential components of the regular human diet. Numerous evidence of health and nutritional benefits from the consumption of fresh fruits and vegetables have been documented in the literature *(8, 11, 15).* According to most agricultural economists, sales of fresh-cut produce have increased sharply from approximately $3 billion in 1994 to $12.5 billion in 2004 *(32).* The U.S. Department of Agriculture (USDA) has recently emphasized the need for consumption of fresh produce by recommending at least five daily servings in the diet *(5).*

In recent years, the sales of organic foods have increased at an annual average rate of 20% in the United States, and most estimates indicate that the market expansion for organic foods will continue at the same rate for the next 5 years *(7, 24).* As much as 42% of the organic food sold is organic produce, and 93% of this produce is in the form fresh fruits and vegetables *(7).* The USDA Organic Rule implemented in 2002 included the acceptable production practices for foods marketed as organic, which largely limited the use of vegetable crop fertilizers to animal and plant wastes *(23).*

In recent years, the number of foodborne outbreaks caused by contaminated fresh fruits and vegetables has increased sharply. Contaminated produce currently accounts for 12% of foodborne illnesses and 6% of foodborne outbreaks in the United States compared with 1 and 0.6%, respectively, in the 1970s *(6).* Sivapalasingam et al. *(29)* suggested that the majority of produce-related foodborne outbreaks for which the etiological agent(s) were identified were caused by pathogenic bacteria. In the last 10 to 15 years, *Salmonella* and *Escherichia coli* O157:H7 have been the two most common etiological agents responsible for produce-related outbreaks in this country. Researchers have documented outbreaks of *Salmonella* and *E. coli* O157:H7 infection from produce consumption in various states in the United States, including Minnesota *(10, 19).*

Because organic growers rely primarily on animal manure for fertilization of their soil, it has been suggested that organically grown foods have a greater risk of pathogenic contamination than do their conventional counterparts *(31).* However, there are very few published studies that have included microbial risk assessment of organic fruits and vegetables. In our previous study, the prevalence of *E. coli* in certified organic produce at the preharvest stage was greater than that in conventional counterparts, but this difference was not statistically significant *(22).* Also in that study, none of the preharvest certified organic and conventional produce tested positive for either *Salmonella* or *E. coli* O157:H7 and only two semiorganic samples were con-

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taminated with *Salmonella.* In another study, similar levels of *E. coli* contamination were found in organic and conventional spring salad green mix, and all samples were negative for *Salmonella* and *Listeria monocytogenes (25).* However, the issue of whether organic produce poses a greater risk for foodborne disease remains largely unresolved.

Few reported surveys have focused on microbial quality of organic produce. All of these studies were focused on retail and postharvest samples of organic vegetables, and no pathogenic bacteria such as *Salmonella* and *E. coli* O157:H7 were found in organic produce *(16, 18, 27).* Outbreaks of infection from foodborne pathogens in contaminated organic produce have not been documented in the United States. Collection of more data on the comparative microbiological quality and safety of organic and conventional produce at farms would greatly enhance our understanding of factors that contribute to contamination of fresh fruits and vegetables.

This extensive longitudinal study was undertaken to determine the microbiological quality of fruits and vegetables collected at the preharvest stage during two consecutive harvest seasons and to assess the effect of type of farm, produce variety, and harvest season on the coliform counts, *E. coli* prevalence, and the presence of *Salmonella* and *E. coli* O157:H7.

MATERIALS AND METHODS

Classification of farms. Farmers that grew fresh fruits and vegetables were invited by telephone or personal contact to participate in this study. All participating farms were located in Minnesota and Wisconsin. The farmers participated by allowing the collection of fruits and vegetables from their cultivation plots before harvest. Farms were separated into three major categories: organic, semiorganic, and conventional. Organic farms were those that were currently certified by a USDA-accredited organic certification agency. Semiorganic farms were those that reported using organic practices but were not certified. Conventional farms were those operations that could use any type of farming practice. Participation from organic farmers was lower in the second year of the study than in the first year. Communications with organic farmers in Minnesota and Wisconsin indicated that the main reason for the drop in participation was that some of the organic growers did not renew their certification. Most of the organic farmers in the upper midwestern states of Minnesota and Wisconsin are small-scale family farmers, and some of them were not interested in providing the necessary paperwork and fees associated with the renewal process.

Sampling of fresh fruits and vegetables. The participating farms were visited two or three times during June, July, August, and September in 2003 and 2004. Samples of fruits and vegetables were collected directly from the farm fields and were transferred into sterile zip-lock bags without being washed or having soil particles rubbed off. Gloves and knives were sanitized with alcohol swabs between collections to prevent cross-contamination. Produce types were lettuces, leafy greens, cabbages, peppers, tomatoes, berries, broccoli, summer squash, cucumber, zucchini, and other types of produce in small numbers, e.g., bok choy, cantaloupe, apple, kohlrabi, sprouts, and peas. Lettuce types were Romaine, head lettuce, and leaf lettuce. Leafy greens were spinach, kale, collards, Swiss chard, and mixed. Cabbages collected were Chinese and red. Pepper types were bell, banana, yellow, and red. Tomato types were Roma, cherry, and beefsteak. Berries were strawberries, raspberries, and blueberries.

The sample amounts varied from one produce type to another. For leafy greens such as spinach, kale, and collards, about 500 g of leaves was collected from different plants in a portion of a field of cultivation, and those leaves constituted one sample. For cucumber, summer squash, pepper, and tomato, two to five of these vegetables collected from different areas of a particular location in a field constituted sample. For cantaloupe, cabbage, bok choy, and head lettuce, one head of lettuce, cabbage, or bok choy or one cantaloupe constituted a sample. For berries, about 500 g of fruits ripe enough for harvest was collected from different plants in a particular location of a field and placed in sterile plastic boxes. To ensure representative sampling from a field of cultivation, produce from different locations of a field were sampled. Most of the produce fields were sampled in triplicate, and for large fields as many as five samples were collected from different locations.

Sample bags and boxes were marked with produce type, farm identity, sample number, and date of collection, placed in insulated coolers with ice packs, and sent to the laboratory. Samples were received within 10 h of collection and were stored at 4°C in insulated coolers or cardboard boxes until microbiological analyses began, which was within 48 h of sampling.

Microbiological analyses. Microbiological analyses of fruits and vegetables started with sample preparation, which was specific for sample type. For leafy greens such as lettuce, spinach, kale, collards, cabbage, and bok choy, leaves from the outside and inside sections were chosen for a 25-g sample. For produce types such as summer squash, zucchini, cucumber, tomato, and pepper, one or two of these vegetables were cut into small pieces, and pieces from different portions of the vegetable were chosen for a 25-g sample. For fruits such as raspberries and strawberries, two to six fruits (depending on the size of each fruit) were chosen from different locations of the sample box to constitute a 25-g sample. Each 25-g produce sample was transferred into 225 ml of appropriate enrichment medium such as lauryl sulfate tryptose broth (LST; Neogen, Inc., Lansing, Mich.), modified *E. coli* broth (EC; Neogen), and universal preenrichment broth (UPB; 5 g liter^{-1} tryptone, 5 g liter^{-1} proteose peptone, 15 g liter^{-1} KH_2PO_4, 7 g liter^{-1} Na_2HPO_4, 5 g liter^{-1} NaCl, 0.5 g liter^{-1} glucose, 0.25 g liter^{-1} $MgSO_4 \cdot 7H_2O$, 0.1 g liter^{-1} ferric ammonium citrate, and 0.2 g liter^{-1} sodium pyruvate) in sterile stomacher bags. The sample was then mixed with the enrichment broth in a stomacher (Tekmar Co., Cincinnati, Ohio) for 2 min.

Coliform and *E. coli* counts were determined with the three-tube most-probable-number (MPN) system using three 10-fold dilutions in 9-ml tubes of LST broth. The LST tubes were incubated for 24 and 48 h at 37°C, and those showing growth and gas production were transferred to 9 ml of brilliant green bile (BGB; Neogen) broth tubes that each contained a Durham's tube. The BGB broth tubes were incubated at 37°C for 24 and 48 h for selective enrichment of coliforms. Broth cultures from the tubes showing both growth and gas production were streaked on eosin methylene blue (EMB; Neogen) plates. The EMB plates were incubated for 24 h at 37°C and then examined for characteristic *E. coli* colonies that had a dark center with or without a greenish metallic sheen. The identity of suspected *E. coli* colonies was confirmed by indole, methyl red, Voges Proskauer, and citrate fermentation tests. Predominant coliform types were determined by identifying the isolated colonies from the highest dilution of the sample on EMB plates with Analytical Profile Index (API 20E) strips (bioMérieux, Marcy l'Etoile, France). The detection limit for this method was 10 CFU g^{-1}.

For detection of *E. coli* O157:H7, 25 g of sample was blended with 225 ml of EC broth supplemented with novobiocin (ICN Biomedicals, Inc., Irvine, Calif.) by adding 2.25 ml of a 2 g $liter^{-1}$ novobiocin stock solution. This enrichment was incubated without shaking at 35°C for 24 h *(9)*. After incubation, 1 ml of the enrichment culture was mixed with 20 μl of a suspension of magnetic beads (Dynal ASA, Oslo, Norway) coated with anti-O157 antibody and incubated at room temperature for 30 min with gentle shaking. After incubation, the tubes were placed in a strong magnetic field (Miltenyi Biotech, Inc., Auburn, Calif.) for 5 min to separate the beads, and the liquid was discarded. The beads were then resuspended in 1 ml of buffered peptone water (BPW; 10 g $liter^{-1}$ peptone, 5 g $liter^{-1}$ NaCl, 3.5 g $liter^{-1}$ Na_2HPO_4, and 1.5 g $liter^{-1}$ NaH_2PO_4) that contained 0.05% Tween 20 (ICN Biomedicals) and incubated with gentle shaking for 5 min at room temperature, and the tubes were placed back into the magnetic field for 5 min. The liquid was discarded, and this washing step was repeated two more times. After the final wash, the beads were resuspended in 100 μl of BPW and plated on sorbitol MacConkey agar (Neogen) supplemented with 2.5 mg $liter^{-1}$ potassium tellurite and 0.05 mg $liter^{-1}$ cefixime (Sigma, St. Louis, Mo.). Colorless or pale colonies were tested for O157 antigen using an *E. coli* O157:H7 latex agglutination test kit (Oxoid, Ltd., Hampshire, UK). *E. coli* O157:H7 ATCC 43895 was used as the positive control, and this analytical method of detection was validated for detection of 10 *E. coli* O157:H7 cells per 25 g of lettuce (data not shown).

Detection of *Salmonella* was conducted using the standard official technique described in the U.S. Food and Drug Administration (FDA) *Bacteriological Analytical Manual (1)* with minor modifications. Each 25-g produce sample was blended with 225 ml of UPB, and the enrichment was incubated for 18 to 24 h at 35°C *(13)*. The pH of enrichments for acidic fruits such as strawberry and raspberry did not fall below 6.0. After incubation, 1.0 ml of the preenriched sample was transferred to 9 ml of tetrathionate (TT) broth (Difco, Becton Dickinson, Sparks, Md.) and 9 ml of Rappaport Vassiliadis (RV) broth (Difco, Becton Dickinson). The TT and RV broth tubes were incubated at 37 and 42.5°C, respectively, for 24 h. Cultures showing distinct turbidity were then streaked onto xylose lysine desoxycholate (Neogen) and bismuth sulfite (Difco, Becton Dickinson) plates with a 25-μl inoculation loop, and the plates were incubated at 37°C for 18 to 24 h. Suspected *Salmonella* colonies were transferred onto lysine iron agar (LIA) and triple sugar iron (TSI) slants (Difco, Becton Dickinson) by streaking and stabbing. The LIA and TSI tubes were incubated at 35°C for 24 h, and suspected colonies that produced the characteristic biochemical reactions were confirmed with the Salmonella-Tek ELISA test system (bioMérieux). *Salmonella* Typhimurium ATCC 14028 was used as the positive control, and this analytical procedure was validated for detection of as few as 10 *Salmonella* cells per 25 g of lettuce (data not shown).

Statistical analyses. The average coliform and *E. coli* counts were recorded in logarithmic scale, and these counts were compared among the various produce types and the three different farm types using Student's *t* test *(20)*. Percent prevalence of *E. coli* among various produce types and produce samples collected from the three farm types were compared using standard and multiple chi-square tests *(21)*. Differences in counts and prevalences were considered significant at $P < 0.05$.

TABLE 1. *Number of participating farms and produce samples collected*

	Season:					
	2003		2004		Total	
Farm type	Samples	Farms	Samples	Farms	Samples	Farms
Organic	178	14	295	8	473	14
Semiorganic	372	24	539	24	911	30
Conventional	297	19	348	14	645	19
Total	847	57	1,182	46	2,029	63

RESULTS

The organic, semiorganic, and conventional farms that took part in this 2-year study were located in the upper midwestern states of Minnesota and Wisconsin. A total of 24 semiorganic farmers participated in each of the 2003 and 2004 harvest seasons by allowing sampling of fruits and vegetables directly from their fields (Table 1). Fourteen organic and 19 conventional farms participated in 2003, but only 8 organic and 14 conventional farms participated in 2004. In 2004, several organic farms in the area decided not to renew their certification documents, and some organic farms stopped growing produce. These uncontrollable events resulted in a smaller number of organic farms participating in the study in 2004.

A total of 2,029 samples of fresh fruits and vegetables were collected and analyzed in 2003 and 2004 (Table 1). Three hundred thirty-five more samples were collected in 2004 than in 2003, representing a 39% increase. In 2003, the average number of produce samples per farm was 16 for the semiorganic and conventional farms and 13 for organic farms. In 2004, the average number of samples per farm was 22, 37, and 25 for the semiorganic, organic, and conventional farms, respectively. The four major produce types that contributed approximately 50% of the total number of samples were leafy greens, cabbages, peppers, and tomatoes. Other major types of produce were berries, cucumber, zucchini, lettuces, summer squash, broccoli, bok choy, and cantaloupe. Berries were strawberries, raspberries, and blueberries. Other produce types collected and analyzed in smaller numbers were melon, kohlrabi, green beans, sprouts, and peas. The produce types responsible for increasing the number of samples in 2004 were leafy greens (62% more), peppers (74% more), tomatoes (42% more), and berries (81% more). The produce and farm types responsible for increasing the number of samples in 2004 were organic leafy greens (47 more samples), semiorganic berries (46 more samples), organic peppers (32 more samples), semiorganic leafy greens (30 more samples), semiorganic tomatoes (29 more samples), and organic tomatoes (26 more samples).

The percentages of each of the different produce types in the total sample lot differed among produce types and among the three farm types (Table 2). During the two sampling seasons, leafy greens accounted for more than 16% of the organic and semiorganic samples but never for more than 4% of the conventional samples. Peppers contributed

TABLE 2. *Percent distribution by produce type of samples collected in 2003 and 2004 from organic, semiorganic, and conventional farms*

	Farm type:							
	Organic		Semiorganic		Conventional		Total	
Produce type	2003	2004	2003	2004	2003	2004	2003	2004
Berries	10.2	2.4	6.2	12.8	9.8	14.6	8.0	10.7
Bok-choi	1.8	1.0	2.4	1.5	0	0.4	1.4	1.0
Broccoli	8.4	3.0	5.6	7.4	2.0	4.0	4.8	5.3
Cabbages	11.8	9.5	11.6	7.6	9.1	11.8	10.4	9.3
Cantaloupe	0	1.0	2.4	1.1	4.0	1.7	2.4	1.3
Cucumber	6.7	8.5	7.0	6.3	13.8	12.6	9.2	8.7
Leafy greens	16.8	26.2	19.1	18.7	4.0	1.5	13.0	15.5
Lettuces	18.5	7.4	7.8	8.1	4.0	3.4	8.5	6.5
Others[a]	0	4.1	2.2	2.2	2.0	4.1	4.3	3.2
Peppers	6.7	14.9	12.4	13.9	15.2	17.2	11.8	15.2
Summer squash	5.1	3.4	5.1	6.3	5.4	5.7	5.1	5.4
Tomatoes	7.3	13.2	10.4	10.8	15.5	12.9	11.2	12.1
Zucchini	6.7	5.4	7.8	3.3	15.2	10.1	9.9	5.8
Total no. of samples	178	295	372	539	297	348	847	1,182

[a] Apples, kohlrabi, sprouts, and peas.

more to the conventional samples (more than 15%). Significantly more cucumber and zucchini samples were collected from conventional than from semiorganic and organic farms.

Coliform bacteria were detected in approximately 70% of the semiorganic fruits and vegetables in each of the 2 years of sampling. Among the organic produce samples, 84 and 80% were coliform positive in 2003 and 2004, respectively. For conventional produce, 75 and 64% were coliform positive in 2003 and 2004, respectively. For the 2 years of this study, the average coliform counts for all of the farm types ranged from 1.5 to 2.3 log MPN/g (Table 3). In 2004, conventional produce had significantly lower coliform counts than did organic and semiorganic produce, but in 2003 this difference was significant only for semiorganic samples. From a subset of 826 samples positive for coliforms, *Enterobacter* spp. were detected in 58% of the samples and *Klebsiella* spp. were detected in 28% of the samples. *Enterobacter cloacae* and *Klebsiella oxytoca* were the two predominant coliform species.

Among the produce samples that had detectable coliforms, the average counts ranged from 1.4 log MPN/g among semiorganic and organic berries in 2003 to 4.8 log MPN/g in semiorganic summer squash in 2004 (Table 4). The average coliform counts in leafy greens, cucumber, and tomatoes, did not differ significantly between different farm types or between the two sampling years. In general, produce types such as peppers, tomatoes, and berries had lower average coliform counts of 1.4 to 2.7 log MPN/g, compared with produce types such as leafy greens, lettuces, cabbages, summer squash, zucchini, and cucumber, which had 2.2 to 4.8 log MPN/g. For the nine major produce types, the average coliform count did not show any particular trend for the two sampling years. Conventionally grown produce had either significantly lower or similar average coliform counts compared with their semiorganic and organic counterparts. However, in 2004 conventional berries had significantly higher average coliform counts than did semiorganically grown peppers and berries.

The majority of the produce samples did not have detectable *E. coli* contamination. In the 2 years of sampling, 68 semiorganic samples (8%) and 34 organic fruit and vegetable samples (7%) had detectable *E. coli* contamination. For conventional produce, as many as 13 samples (2%) tested positive for *E. coli.* Among the 24 participating semiorganic farms, 11 farms in 2003 and 14 in 2004 had at least one *E. coli*–positive produce sample (Fig. 1). Four of the 14 organic farms in 2003 and 7 of the 8 organic farms in 2004 had at least one *E. coli*–positive sample. Among

TABLE 3. *Overall coliform and* E. coli *counts in produce from three farm types in 2003 and 2004*[a]

	Coliform counts (log MPN/g)		*E. coli* counts (log MPN/g)	
Farm type	2003	2004	2003	2004
Organic	2.1 ± 0.2 AB	2.3 ± 0.1 A	2.0 ± 0.3	2.3 ± 0.3
Semiorganic	2.3 ± 0.1 A	2.3 ± 0.1 A	2.3 ± 0.2	2.4 ± 0.2
Conventional	2.0 ± 0.1 B	1.5 ± 0.1 B	2.4 ± 0.6	2.0 ± 0.2

[a] Values are mean ± standard error. Within columns, coliform counts with different letters are significantly different ($P < 0.05$). *E. coli* counts without letters are not significantly different.

TABLE 4. *Coliform counts for major produce types from organic, semiorganic, and conventional farms in 2003 and 2004*

Produce type	Coliform counts (log MPN/g)					
	Organic		Semiorganic		Conventional	
	2003	2004	2003	2004	2003	2004
Berries	1.4 ± 0.1 A	2.0 ± 0.2 AB	1.4 ± 0.1 A	1.7 ± 0.2 AB	1.9 ± 0.2 AB	2.2 ± 0.2 B
Cabbages	3.8 ± 0.4 A	3.8 ± 0.3 A	3.3 ± 0.4 AB	3.4 ± 0.4 AB	2.7 ± 0.2 B	3.2 ± 0.4 AB
Cucumber	2.2 ± 0.2	2.6 ± 0.3	2.8 ± 0.4	2.5 ± 0.3	2.7 ± 0.1	2.3 ± 0.1
Leafy greens	2.7 ± 0.4	2.7 ± 0.2	3.6 ± 0.3	3.5 ± 0.2	3.0 ± 0.4	2.4 ± 0.4
Lettuces	3.1 ± 0.2 A	4.1 ± 0.1 A	3.2 ± 0.4 A	3.9 ± 0.5 A	1.5 ± 0.2 B	3.2 ± 0.5 A
Peppers	1.8 ± 0.1 AB	1.9 ± 0.2 ABC	1.8 ± 0.4 AB	2.7 ± 0.6 C	2.3 ± 0.2 BC	1.6 ± 0.1 A
Zucchini	3.3 ± 0.4 AB	4.1 ± 0.8 A	3.8 ± 0.9 AB	3.3 ± 0.8 AB	3.8 ± 0.4 AB	2.9 ± 0.3 B
Summer squash	4.1 ± 0.6 AB	4.1 ± 0.4 AB	3.6 ± 0.9 AB	4.8 ± 0.7 A	2.9 ± 0.4 B	3.1 ± 0.6 B
Tomatoes	2.1 ± 0.2	2.0 ± 0.2	1.9 ± 0.1	1.8 ± 0.2	2.1 ± 0.2	1.8 ± 0.1

[a] Values are mean ± standard error. Within rows, counts with different letters are significantly different ($P < 0.05$). Counts without letters are not significantly different.

the conventional growers, 3 of 19 in 2003 and 5 of 14 in 2004 had at least one *E. coli*–positive produce sample.

The prevalence of *E. coli* on those farms that had at least one contaminated produce sample ranged from 3.7 to 50% in 18 of the 57 participating farms in 2003 and from 1.4 to 50% in 26 of the 46 participating farms in 2004 (Fig. 1). In 2003, the *E. coli* prevalence on semiorganic farms was 4.8 to 50%, and the prevalence in organic farms was within a narrower range of 11 to 33% (Fig. 1A). The prevalence of *E. coli* on conventional farms was never higher than 7% in 2003. In 2004, *E. coli* prevalence on semiorganic farms was 2 to 50% and that on organic farms was 1 to 24% (Fig. 1B). The prevalence on conventional farms in 2004 was within a wider range of 3 to 38% compared with the prevalence on this type of farm in 2003.

Of the 18 semiorganic farms that had at least one *E. coli*–positive produce sample, seven (farms 1, 2, 5, 6, 7, 8, and 9) had this kind of contamination in both 2003 and 2004. Among these farms, farm 5 had 50% *E. coli* prevalence in both years. Farms 8 and 9 had consistent preva-

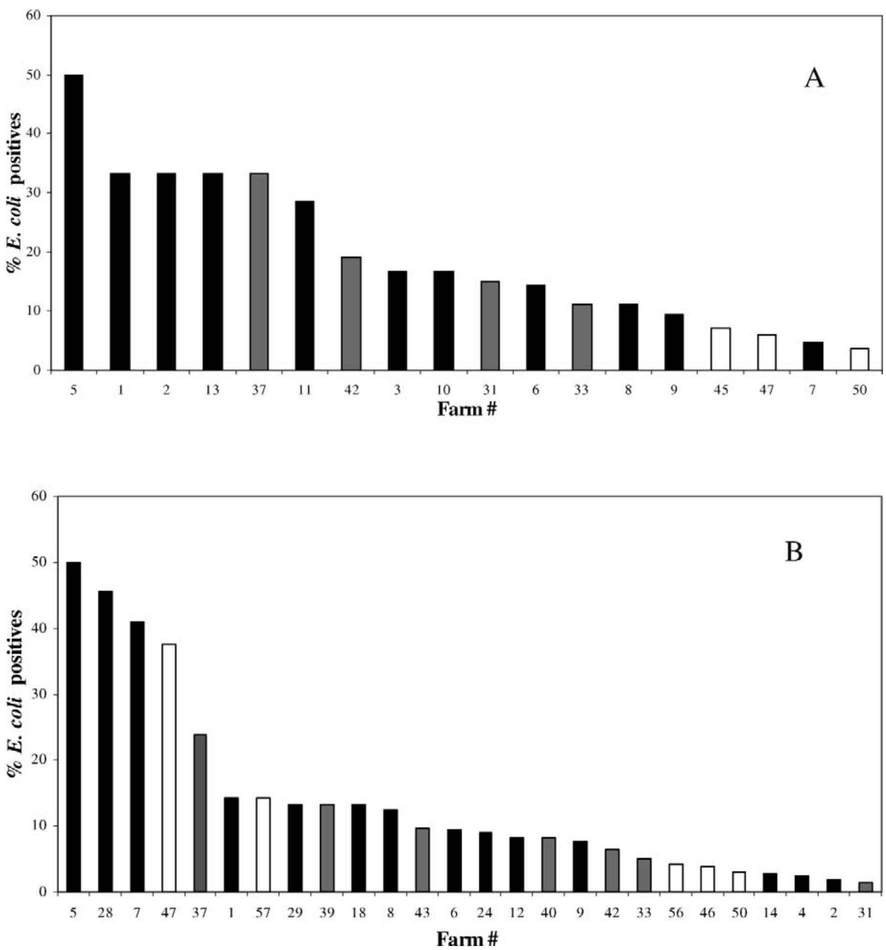

FIGURE 1. *Prevalence of* E. coli *on semiorganic (■), organic (■), and conventional (□) farms from which at least one contaminated sample was collected in 2003 (A) and 2004 (B).*

TABLE 5. *Prevalence of* E. coli *in different produce types from organic, semiorganic, and conventional farms in 2003 and 2004*[a]

	% *E. coli*–positive samples (no. positive/total)					
	Organic		Semiorganic		Conventional	
Produce types	2003	2004	2003	2004	2003	2004
Berries	0 (0/18)	0 (0/7)	0 (0/23)	2.9 (2/69)	0 (0/26)	0 (0/51)
Broccoli	6.7 (1/15)	0 (0/9)	0 (0/21)	5.0 (2/40)	0 (0/6)	0 (0/14)
Cabbages	14.3 (3/21)	3.6 (1/28)	10.0 (4/40)	14.6 (6/41)	3.7 (1/27)	7.3 (3/41)
Cucumber	8.3 (1/12)	12.0 (3/25)	4.3 (1/23)	2.9 (1/34)	0 (0/41)	0 (0/44)
Leafy greens	6.7 (2/30) A	9.1 (7/77) A	23.9 (17/71) B	13.9 (14/101) AB	25.0 (3/12) AB	20.0 (1/5) AB
Lettuces	18.2 (6/33)	22.7 (5/22)	20.0 (7/35)	9.3 (4/43)	0 (0/12)	25.0 (3/12)
Peppers	0 (0/12)	2.3 (1/44)	0 (0/46)	1.3 (1/75)	0 (0/45)	0 (0/60)
Summer squash	0 (0/7)	0 (0/10)	0 (0/19)	2.9 (1/34)	0 (0/16)	5.0 (1/20)
Zucchini	8.3 (1/12)	0 (0/16)	0 (0/29)	0 (0/18)	0 (0/45)	0 (0/35)

[a] For leafy greens, percentages with different letters are significantly different ($P < 0.05$). Percentages in rows without letters are not significantly different.

lence of approximately 11 to 12% and 8 to 9%, respectively, in both the years. Among the organic farms, seven had at least one produce sample that tested positive for *E. coli.* Four of these organic farms (farms 31, 33, 37, and 42) had these indicator bacteria in their produce in both years. The *E. coli* prevalence on these four organic farms decreased 1.4- to 15-fold from 2003 to 2004. Seven conventional farms had at least one *E. coli*–positive produce sample, and farms 47 and 50 had such contamination in both years of this study. On farm 47, the *E. coli* prevalence increased 10-fold from 2003 to 2004, whereas that on farm 50 remained approximately the same.

The prevalence of *E. coli* in samples of leafy greens from semiorganic and conventional farms was as much as threefold higher than that in organic samples. However, only the prevalence in semiorganic leafy greens in 2003 was significantly greater than that in organic greens (Table 5). When the *E. coli* prevalence in leafy greens was compared among the three farm types for both years combined, it was significantly greater in semiorganic (18%) and conventional (24%) leafy greens than in their organic (8%) counterparts ($P < 0.05$). The *E. coli* prevalence in lettuces ranged from 0% in conventional samples in 2003 to 25% in conventional samples in 2004 (Table 5). The prevalence in semiorganic cabbages was approximately two to four times the prevalence in conventional cabbages for both years; however, these differences were not significant. *E. coli* prevalence in organic cucumbers was 8 to 12%, approximately two to four times the prevalence in semiorganic cucumbers; however, this difference was not significant. None of the semiorganic, organic, and conventional tomato, cantaloupe, apple, and kohlrabi samples had *E. coli* contamination in either 2003 or 2004.

Samples of leafy greens and lettuces had the highest prevalence of *E. coli* compared with all other produce types when all three farm types were combined for both years (Fig. 2). Cabbages had a significantly lower *E. coli* prevalence than did lettuces and leafy greens in 2003 and a significantly higher prevalence compared with peppers and berries. When zucchini, summer squash, and cucumber were clustered together as a single produce category, the prevalence of *E. coli* was similar to that observed in peppers and berries. However, that prevalence was significantly lower than that in lettuces, leafy greens, and cabbages. Within each category of produce, the *E. coli* prevalence was not significantly different between the two sampling years.

Fresh produce that was contaminated with *E. coli* had counts of approximately 2.0 to 2.4 log MPN/g (Table 3). Both semiorganic and organic produce samples had wide ranges of 1.0 to 6.3 and 0.6 to 7.8 log MPN/g, respectively. The range of *E. coli* counts in conventional produce samples were within a narrower range of 1.4 to 4.0 log MPN/g. Among the major produce types, lettuces, leafy greens, and cabbages had *E. coli* counts of 2.2 to 2.4 log MPN/g. For all other produce types with *E. coli* contamination, the average count was 1.9 log MPN/g. However, these differences in counts of the indicator bacteria among produce types or farms types were not significant.

All of the 2,029 fruit and vegetable samples collected during the 2003 and 2004 seasons were tested for the presence of *Salmonella* and *E. coli* O157:H7. Neither of these two pathogenic bacteria were detected in any of the produce samples.

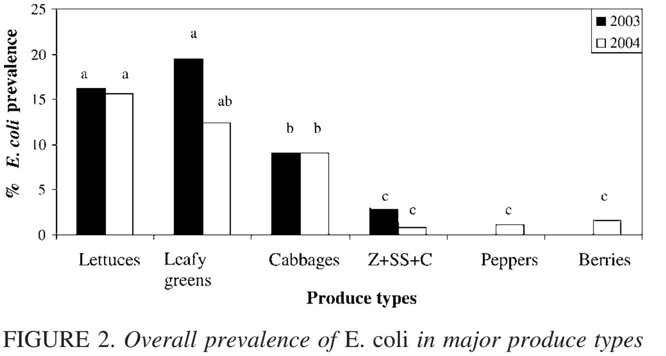

FIGURE 2. *Overall prevalence of* E. coli *in major produce types for the two sampling years. Prevalence was compared between produce types; produce types with different letters were significantly different* (P < 0.05). *Peppers and berries had zero* E. coli *prevalence in 2004. Z+SS+C, zucchini, summer squash, and cucumber combined.*

DISCUSSION

The present study was conducted to generate data on the microbiological quality of fresh fruits and vegetables at the preharvest stage. The safety of fresh fruits and vegetables has been an area of increasing concern as the number of foodborne outbreaks and illnesses from consumption of contaminated produce has increased significantly in recent years *(29)*. Many pathogenic microorganisms are natural inhabitants of the gastrointestinal tract of livestock, and animal manure has been identified as a vehicle for transmission of these pathogens to foods. Because animal manure is one of the few fertilizers available to organic farmers, researchers have speculated that the increase in foodborne diseases could also be linked to the increase in the consumption of fresh organic vegetables. We conducted an initial study based on a smaller group of samples during one harvest season in 2002 *(22)*. The present research involved a more extensive survey of more farms from the three farm types and included 2 years of sampling and more than 2,000 samples of fruits and vegetables collected directly from farm fields.

Coliforms are members of the *Enterobacteriaceae*. Because they are considered to be naturally present in fruits and vegetables, they can be used to assess the extent of bacterial populations *(23)*. These bacteria were detected in 92% of all the samples analyzed in our previous study *(17, 22)*. This percentage is significantly higher than the percentage of positive samples detected in the present study (72%) ($P < 0.05$). Sagoo et al. *(28)* reported that three-fourths of almost 4,000 samples of retail ready-to-eat salads contained *Enterobacteriaceae*. The average coliform counts in positive samples were 1.5 to 2.5 log MPN/g (Table 3), which are slightly lower than the average counts determined previously. In a recent study, similar coliform counts (1.5 to 3.0 log CFU/g) were found in preharvest samples of spinach, cilantro, mustard greens, parsley, and cantaloupe *(14)*. Our results indicate that on average, coliform populations on fresh fruits and vegetables are very consistent over the years and that coliform loads may differ significantly among various produce types.

In one study of conventional and organic spring salad green mix from a produce processor in California, average coliform counts were 2.75 and 2.97 log CFU/g, respectively *(25)*. These counts are consistent with the 2.4 to 3.6 log MPN/g that we found in organic, semiorganic, and conventional leafy greens (Table 4). The predominant coliform bacteria in fresh fruits and vegetables have been documented in different studies. *Enterobacter* and *Klebsiella* were two major coliform types identified as natural bacterial flora in lettuce *(30)*. In our preliminary report, we identified *Enterobacter sakazakii* and *E. cloacae* as the two predominant coliform species in preharvest samples *(22)*. In the present study, in a subset of fruit and vegetable samples, the predominant coliform species were *E. cloacae* and *K. oxytoca*. These observations indicate that these two genera of coliforms are frequently present as part of the natural bacterial flora of fruits and vegetables *(17, 26)*.

Few surveys have included evaluation of the presence of pathogenic bacteria in fresh fruits and vegetables. The FDA conducted a large survey of 1,028 domestically produced fruits and vegetables and reported that only 0.5% were contaminated with foodborne pathogens *(4)*. Three of 93 green onion samples were contaminated with *Shigella*, and *Salmonella* was detected in 1 of 142 lettuce samples and 1 of 85 cilantro samples. None of these produce samples had detectable *E. coli* O157:H7 contamination. The FDA also conducted a similar survey of imported fruits and vegetables that included approximately 1,000 samples *(3)*. In that survey, as high as 2.2% of the samples were contaminated with *Salmonella* and *Shigella*. Among the various types of imported produce included in that survey, leafy greens such as cilantro, lettuce, celery, and green onions were tested positive for these two pathogens. In another large survey of 3,825 bagged ready-to-eat salad vegetables collected from retail stores in the United Kingdom *(28)*, most (99.3%) of the samples had no detectable foodborne pathogens such as *Salmonella, E. coli* O157:H7, *L. monocytogenes*, and *Campylobacter*, and only five samples were contaminated with *Salmonella*. In a more recent report, *Salmonella* was detected in three cantaloupe samples from a total of 199 samples of leafy greens, 109 herbs, and 90 cantaloupes *(14)*.

Contamination of organic fruits and vegetables with pathogenic bacteria has been rarely reported in the literature. Two studies of organic produce at the retail level in the United Kingdom included 86 samples of tomato, carrot, mushroom, cherry pepper, and alfalfa sprouts and 3,200 samples of broccoli, cabbage, celery, lettuce, radish, and others *(18, 27)*. Neither of those studies revealed contamination with *Salmonella* or *E. coli* O157:H7. In a more recent study in Norway, *Salmonella* and *E. coli* O157:H7 were not detected in 179 organically grown lettuce samples collected from 12 producers *(16)*. In our previous study, *E. coli* O157:H7 contamination was not detected in any of the organic and conventional produce *(22)*. However, one lettuce and one pepper sample had *Salmonella* contamination.

The use of *E. coli* as an indicator of fecal contamination has been questioned by a number of researchers, who have found that this bacterium can be naturally present and is capable of growing in certain soil environments *(2, 12)*. Ishii et al. *(12)* reported that the population of ''naturalized'' *E. coli* in the soil could reach 10^5 CFU/g in northern temperate areas during the warmer months. Those findings suggest that some of the *E. coli*–positive samples detected in this study could have been contaminated with indigenous soil strains and that their presence might not necessarily indicate exposure of produce to fecal material.

In the present study, those fruits and vegetables that tested positive for *E. coli* had average counts that ranged from 2 to 2.4 log MPN/g (Table 3). Among those *E. coli*–positive samples, the average counts were not significantly different among the various produce types or the three farm types. Mukherjee et al. *(22)* reported an average *E. coli* count of 3.1 log MPN/g in preharvest produce samples. They also found that the average counts of this indicator bacterium did not differ significantly among produce types and farm types. Loncarevic et al. *(16)* reported an average

E. coli count of 1.5 log CFU/g in contaminated organic lettuce samples. Another research group reported average *E. coli* counts in produce such as leafy greens, herbs, and cantaloupes of less than 1 to 2.5 log CFU/g *(14)*. These reported findings for *E. coli* populations in fresh produce are similar to the average *E. coli* counts of 2 to 2.4 log MPN/g observed in the present study.

In the 2 years of this study, nine farms had more than 30% prevalence of *E. coli* contamination in their produce, and seven of these farms were semiorganic. Except for one farm in each of 2003 and 2004, none of the organic farms had more than 20% *E. coli* prevalence. Among the conventional farms in 2003, the farm prevalence never reached 10% and only one conventional farm had more than 20% *E. coli* prevalence throughout this study. Unlike in our previous study, the data analyses in the present study did not involve clustering results from organic (''certified organic'' in the previous report) and semiorganic (''non-certified organic'') farms. Despite the fact that the overall proportion of *E. coli*–positive samples was higher for organic (7%) and semiorganic (8%) farms than for conventional farms (2%), we did not consider it appropriate to conduct a statistical analysis and draw any conclusions from this difference because of the disproportionately smaller number of produce types that appear to be more susceptible to contamination such as lettuces and leafy greens collected from conventional operations. Instead, we focused our comparisons among farm types by produce type (Table 5). A follow-up study will include an investigation of the risk factors associated with *E. coli* contamination for each of the farms and produce varieties.

When the *E. coli* prevalence in different produce types were compared among the three farm types, prevalences were significantly different only for leafy greens on organic and semiorganic farms (Table 5). Thus, the major difference in *E. coli* prevalence in the present study was observed between various produce types (Fig. 2). The presence of visible soil particles between the leaves in lettuces, leafy greens, and cabbages might have resulted in significantly higher *E. coli* prevalence in these produce types compared with other types such as zucchini, summer squash, cucumber, peppers, and berries.

In a more recent study, 179 samples of ready-for-sale organic lettuce were collected from organic growers in Norway *(16)*. The *E. coli* prevalence of 8.9% was significantly lower than the approximately 20% prevalence in organic lettuce samples observed in the present study. In another study, none of the 86 samples of organic vegetables, mostly from supermarket chains, were contaminated with *E. coli* *(18)*. The prevalences of *E. coli* reported at the retail level in these studies were smaller than those we found in fresh fruits and vegetables at the farms. This difference was most likely due to the fact that retail ready-to-eat produce samples were washed and sanitized, whereas preharvest produce samples in the present study were not subjected to any treatment.

The results of this study confirmed our previous findings that organic fruits and vegetables do not appear to be more susceptible to preharvest contamination by *Salmonella* and *E. coli* O157:H7 than does conventional produce. For some of the fruit and vegetable types, conventional produce had significantly lower coliform counts than did semiorganic and organic produce. However, the type of farm operation appeared to have little influence on the prevalence of contamination with *E. coli* by produce type. The presence of *E. coli* in produce was not different between the 2 years of the study. Produce types such as leafy greens, lettuces, and cabbages appeared to be more susceptible to *E. coli* contamination on all three types of farms in both years. This finding suggests that if foodborne pathogens were present under similar environmental conditions, leafy vegetables would be a likely vehicle of transmission.

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