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Preharvest Evaluation of Coliforms, *Escherichia coli, Salmonella,* and *Escherichia coli* O157:H7 in Organic and Conventional Produce Grown by Minnesota Farmers

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ABSTRACT

Microbiological analyses of fresh fruits and vegetables produced by organic and conventional farmers in Minnesota were conducted to determine the coliform count and the prevalence of *Escherichia coli, Salmonella,* and *E. coli* O157:H7. A total of 476 and 129 produce samples were collected from 32 organic and 8 conventional farms, respectively. The samples included tomatoes, leafy greens, lettuce, green peppers, cabbage, cucumbers, broccoli, strawberries, apples, and seven other types of produce. The numbers of fruits and vegetables was influenced by their availability at participating farms and varied from 11 strawberry samples to 108 tomato samples. Among the organic farms, eight were certified by accredited agencies and the rest reported the use of organic practices. All organic farms used aged or composted animal manure as fertilizer. The average coliform counts in both organic and conventional produce were 2.9 log most probable number per g. The percentages of *E. coli*–positive samples in conventional and organic produce were 1.6 and 9.7%, respectively. However, the *E. coli* prevalence in certified organic produce was 4.3%, a level not statistically different from that in conventional samples. Organic lettuce had the largest prevalence of *E. coli* (22.4%) compared with other produce types. Organic samples from farms that used manure or compost aged less than 12 months had a prevalence of *E. coli* 19 times greater than that of farms that used older materials. Serotype O157:H7 was not detected in any produce samples, but *Salmonella* was isolated from one organic lettuce and one organic green pepper. These results provide the first microbiological assessment of organic fruits and vegetables at the farm level.

Organic food is one of the fastest growing segments in the food supply, and it has been estimated that organic production will expand at an average rate of 24% during the medium term *(7).* From 1992 to 1997, organic cropland in the United States doubled, and between 1990 and 2002, organic food sales in the United States increased from $1 to $9 billion *(11).* Fresh fruits and vegetables accounted for approximately 40% of the total sales of organic food products in 2001 *(25).* In Minnesota, where profitability on traditional commodities can be marginal and agricultural land costs high, the organic production of fresh fruits and vegetables, in which high-value crops can be grown on relatively small acreages, offers a potential niche for both existing and new farmers. The consumer demand for organic produce is driven largely by the assurance that no synthetic pesticides and fertilizers are used in its production.

According to the recently issued U.S. Department of Agriculture (USDA) organic rule, organically grown fruits and vegetables can be fertilized with natural sources of nutrients such as animal manure, plant debris (green manure), fish emulsion, and kelp. The foodborne pathogens that farm animals may carry in their gastrointestinal tracts can be spread to crops and the environment via manure. Because animal manure is a commonly used fertilizer in organic vegetable production, it has been suggested that organic fruits and vegetables might contain higher levels of pathogenic bacteria than conventional ones. The national organic standards only allow the use of animal manure if it has been composted according to specific procedures or if it is applied more than 90 days before harvesting *(1).* Conventional crops, however, may also be fertilized with manure.

Composting is traditionally intended to control soilborne plant diseases and other pests; if the process is properly conducted, it can also kill foodborne pathogens. A number of studies have demonstrated that composting reduced bacterial populations of *Salmonella, Escherichia coli,* and *E. coli* O157:H7 in manure *(16, 19, 36),* but this effect is dependent on composting time and temperature. The results from these studies suggest that the recommended temperatures required by the USDA would be sufficient to control *Salmonella* and *E. coli* O157:H7 in composted manure.

In recent years, an increasing number of gastrointestinal disease outbreaks have been linked to the consumption of fresh fruits and vegetables. Between 1990 and 2001, contaminated fresh produce was linked to a total of 148 outbreaks that accounted for approximately 9% of all foodborne outbreaks *(26).* A wide variety of foodborne pathogens have caused these outbreaks associated with the consumption of fresh produce. The pathogens most frequently linked to produce-related outbreaks include bacteria (*Sal-*

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monella, E. coli), viruses (Norwalk-like, hepatitis A), and parasites (*Cryptosporidium, Cyclospora*) *(33). Salmonella* and *E. coli* O157:H7 are the leading causes of produce-related outbreaks, accounting for 30 and 20%, respectively, of those outbreaks in which the etiological agent was identified *(23).* Because of these outbreaks, fresh fruits and vegetables might pose an increased food safety risk because they are consumed raw and are susceptible to be contaminated by fecal material and soil at the farm.

A number of reports in the popular media have promoted the idea that organic produce poses a greater risk of transmitting foodborne diseases than does conventional produce *(3, 30),* but there is very little epidemiological and scientific evidence that supports these claims. Only one confirmed foodborne outbreak linked to the consumption of organic vegetables has been reported *(35).* In addition, very few published studies have been conducted to determine the presence of pathogenic bacteria in organic produce, and two of these surveys found no *E. coli* O157:H7 or *Salmonella (20, 24).* Furthermore, all of the published surveys have analyzed fruits and vegetables only at the retail level.

The present study was undertaken to determine the presence of coliforms, *E. coli* (as indicator of fecal contamination), and two foodborne pathogens (*E. coli* O157:H7 and *Salmonella)* in organic and conventional fruits and vegetables produced by farmers in Minnesota and to establish relationships of *E. coli* prevalence with type of produce and farm management practices.

MATERIALS AND METHODS

Farmer recruitment and survey. Farmers were recruited by personal invitation during workshops at the Southwest Research and Outreach Center, by personal visit, and by phone. The participation of farmers was based on their willingness to provide samples and information about their farm practices and their geographical location, i.e., situated in southern and central Minnesota, to allow for the timely collection and transport of samples. Noncertified and certified organic farmers were included in the study because, in this region, the majority of organic vegetable growers were not certified. Grower interviews suggested that noncertified organic vegetable producers, who primarily sell directly to consumers, feel that their customers are already aware of their organic practices, rendering certification unnecessary.

Each farmer was visited once during the winter of 2001 and asked to complete a survey about their production, fertilization, and management practices that were relevant for the 2002 harvest season. The farmers were asked specific questions about their acreage, contact information, certification status and agency of certification (for organic farmers), and fertilization practices, such as the type(s) of manure or compost and/or chemicals, age of manure or compost, and time of application. The questionnaire also requested information on handling practices during harvesting and subsequent handling operations, like washing, packaging, and storage. The information collected from the survey was used to identify associations with the microbiological results.

Sample collection. During May to September 2002, participating farms were visited 2 to 3 times, depending on product availability. The list of vegetables and fruit collected included tomatoes, leafy greens (kale, spinach, amaranth, and Swiss chard), lettuce, green peppers, cabbage, cucumbers, broccoli, strawberries, apples, summer squash, bok choi, zucchini, cantaloupes, carrots, eggplant, raspberries, onions, beets, basil, and kohlrabi. During each visit, 2 to 3 samples of produce that was ready for harvest were picked randomly from different locations on the field and immediately put into sterile zip-lock bags without washing. The sample size for small vegetables was 300 to 500 g. Samples of cabbage, head lettuce, cantaloupes, and bok choi consisted of the entire head or fruit. The samples were then transported in boxes made of insulating material or styrofoam with ice placed on the bottom. Metal or wooden trays separated the ice from the bags containing produce. The temperature of fruits and vegetables was not monitored. The cooler boxes were delivered to the Food Safety Microbiology Laboratory (St. Paul, Minn.) for microbiological analyses within 10 h after collection. Samples were kept stored at 4°C in cardboard boxes or on metal shelves of a walk-in cooler until the analyses began.

Microbiological analysis. Microbiological analyses started within 72 h of receipt of samples. For lettuce, cabbage, and leafy greens, representative leaves from the exterior and interior sections were used to make up the 25-g samples. Twenty-five grams of sample was mixed in a stomacher (Tekmar Co., Cincinnati, Ohio) for 2 min in 225 ml of any of the enrichment broths (lauryl sulfate tryptose [LST] broth, tryptic soy broth [TSB], or universal preenrichment broth [UPB]). The coliform count was determined by the three-tube most-probable-number (MPN) system using three 10-fold serial dilutions in LST (Neogen, Inc., Lansing, Mich.) broth that were incubated for 48 h at 37°C. LST tubes showing growth and gas production were transferred to tubes that contained 9 ml of brilliant green bile (BGB; Neogen) broth for the selective enrichment of coliforms *(13).* The positive BGB enrichment tubes were streaked on eosin methylene blue (EMB; Difco, Becton Dickinson, Sparks, Md.) plates. Suspected *E. coli* colonies were confirmed by indole, methyl red, Voges Proskauer, and citrate fermentation tests. Predominant coliforms in the fruits and vegetable samples were determined by identifying the isolated colonies from the highest dilution of the samples on EMB plates, using API strips (bioMérieux, Marcy l'Etoile, France).

Analysis of *E. coli* O157:H7 was conducted by blending 25-g samples with 225 ml of TSB (Difco) supplemented with novobiocin (ICN Biomedicals, Inc., Irvine, Calif.) and incubated at 42°C for 6 h *(6).* The pH of enrichment broth mixed with acidic fruits (e.g., strawberries and apples) was verified to be not less than 6.8. At this time, 0.5 ml of TSB cultures were mixed with 10 μl of suspensions of magnetic beads coated with anti-O157 antibodies (Dynal ASA, Oslo, Norway) and incubated with gentle motion for 30 min. Tubes that contained the culture/bead mixtures were placed in a strong magnet (Miltenyi Biotec, Inc., Auburn, Calif.) for 5 min, and the liquid was discarded. The beads were resuspended in 0.5 ml of buffered peptone water (BPW) that contained 0.05% Tween 20, incubated for 5 min with gentle agitation, placed on the magnet for 5 min, and the liquid was removed. These steps were repeated two more times. After the final wash, the beads were resuspended with 100 μl BPW and plated on CHROMagar O157 (CHROMagar Microbiology, Paris, France) agar supplemented with potassium tellurite (ICN Biomedicals). Mauve-colored colonies were tested for O157 antigens by using an *E. coli* O157:H7 latex-agglutination test (Oxoid, Ltd., Hampshire, UK). Strain ATCC43895 was used as a positive control, and it was confirmed that this method could detect as few as 10 cells of *E. coli* O157:H7 per 25-g sample (data not shown).

The detection of *Salmonella* was done by a modification of the standard method described in the Food and Drug Administration's *Bacteriological Analytical Manual (2).* Produce samples (25 g) were blended with 225 ml of UPB (containing 5 g of tryptone,

5 g of proteose peptone, 15 g of KH_2PO_4, 7 g of Na_2HPO_4, 5 g of NaCl, 0.5 g of glucose, 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of ferric ammonium citrate, and 0.2 g of sodium pyruvate per liter) and incubated for 24 h at 37°C *(15)*. The pH of enrichment broth mixed with acidic fruits (e.g., strawberries and apples) was verified to be not less than 6.0. The preenriched sample was then transferred into tubes that contained 9 ml of tetrathionate (Difco) and Rappaport Vassiliadis (Difco) broths and incubated at 42.5°C for 24 h. Tubes that showed distinct turbidity were then streaked on xylose lysine desoxycholate (Difco) and bismuth sulfite (Difco) plates. Suspected *Salmonella* colonies were confirmed by the 1–2 system (BioControl, Inc., Bellevue, Wash.). *Salmonella* serovar Typhimurium strain ATCC 14028 was used as a positive control, and it was confirmed that this method detected as few as 10 cells per 25-g sample (data not shown).

The *E. coli* isolates from fruits and vegetables were tested for the presence of the genes that encode for shiga toxins by a duplex PCR based on a protocol published elsewhere *(31)* as follows. Cell templates were made from 500 μl of liquid culture of the *E. coli* isolates grown overnight in Luria-Bertani broth by boiling for 15 min and centrifugation (3 min at 20,000 × *g*). Two microliters of cell templates was mixed with 48 μl of PCR reaction mixture that contained 45 pmol of Shiga toxin-1 primers (forward, 5′-CAGTTAATGTGGTGGCGAAGG-3′; reverse, 5′-CACCAGACAATGTAACCGCTG-3′) and Shiga toxin-2 primers (forward, 5′-ATCCTATTCCCGGGAGTTTACG-3′; reverse, 5′-GCGTCATCGTATACACAGGAGC-3′), 1 mM each deoxynucleoside triphosphate, 1 U of *Taq* DNA polymerase (Promega, Inc., Madison, Wis.), 5 μl of buffer (10 mM KCl, 10 mmM $NH_4)SO_4$, 20 M Tris-HCl [pH 8.8], 2 mM $MgSO_4$, and 0.1% Trition X-100) 4 mM MgCl, and 160 μM bovine serum albumin. The PCR reaction was preformed using a Robocycler model Gradient 96 (Stratagene, Inc., La Jolla, Calif.) for 2 min at 95°C, then 35 cycles of 94°C for 30 s, 50°C for 1 min, and 65°C for 8 min. The last step was at 65°C for 8 min, and then the samples were held at 4°C until electrophoresis. Fourteen microliters of the PCR reaction was mixed with 12 μl of loading dye. The samples were electrophoresed on a 1.5% agarose gel at 4°C for 1.5 h at 110 V. A 1-kb DNA fragment ladder was used as standard. The gels were stained using a 0.5-μg/ml ethidium bromide solution for 30 min, and band patterns of individual isolates were compared using a Gel-Doc 8000 gel documentation system (UVP, Inc., Upland, Calif.). The presence of *stx1* and *stx2* was determined by PCR products of 348 and 584 bp, respectively. *E. coli* O157:H7 strain ATCC 43895 was used as a positive control and *E. coli* K12 as a negative control.

Data analysis. The average coliform counts were calculated, and statistically significant differences between varieties of fruits and vegetables and between organic and conventional farms were determined using Student's *t* test *(27)*. The prevalence of *E. coli* in produce samples was compared among different produce varieties using the χ^2 test. The same statistical tool was used to compare prevalence of *E. coli* between pairs of categories of farms classified according to their different management and fertilization practices. The criteria for statistical significance was based on a $P < 0.05$. Odds ratios (ORs) were calculated to compare the likelihood of *E. coli* contamination and, hence, the relative risks of contamination between these groups *(5)*.

RESULTS

A total of 32 organic and 8 conventional farmers participated in the study by providing samples and answering questions about their management practices. Among the organic farms, 8 were certified by approved agencies and 24 were not certified by an accredited organic certification agency but reported using organic practices. Most of the conventional farms reported approximately 20 acres of land under production, and one farm had a significantly larger operation of 200 acres. Among the organic farms that reported acreage, the average size of operation was approximately 10 acres. No information regarding source of irrigation water, surrounding land use, and acreage per crop was obtained from the farmers. All of the organic farms used aged or composted animal manure regularly as a main source of fertilizer, and four conventional farmers reported using composted manure in addition to their chemical fertilizer. Some of the farmers did not answer all questions in the survey.

TABLE 1. *Distribution of organic and nonorganic samples, according to produce varieties*

Produce varieties	% of samples (no. of samples)		
	Organic	Conventional	Total
Tomato	19.3 (92)	12.4 (16)	17.8 (108)
Leafy greens	17.6 (84)	3.1 (4)	14.5 (88)
Lettuce	10.2 (49)	4.7 (6)	9.1 (55)
Green pepper	9.0 (43)	9.3 (12)	9.1 (55)
Cabbage	8.2 (39)	11.6 (15)	8.9 (54)
Cucumber	6.5 (31)	7.0 (9)	6.6 (40)
Broccoli	6.3 (30)	4.6 (6)	6.0 (36)
Summer squash	3.8 (18)	4.6 (6)	4.0 (24)
Zucchini	1.5 (7)	11.6 (15)	3.6 (22)
Bok choi	3.2 (15)	2.3 (3)	3.0 (18)
Apple	2.1 (10)	4.6 (6)	2.6 (16)
Onion	1.9 (9)	5.4 (7)	2.6 (16)
Strawberry	1.7 (8)	2.3 (3)	1.8 (11)
Other produce	8.6 (41)	16.3 (21)	10.2 (62)

Of 27 organic farmers using manure or compost, 18 reported using material that had been aged 6 to 12 months, 6 farmers waited more than 1 year before application, and only 2 used material that was less than 6 months old. Fourteen of 23 organic farmers applied manure or compost in the spring, 5 in the fall, 3 in both seasons, and only 1 in the summer. The distribution of the type of manure used by 31 farmers either alone or in combination was as follows: chicken 15, cattle 12, sheep 8, and horse 4. As many as seven farmers applied a mixture of three types of manure, and nine used two different animal manures the same year. Among conventional farmers, two reported using cattle manure and one reported using 8-year-old compost.

The organic and conventional farmers provided 476 (117 from certified farms) and 129 produce samples, respectively, for microbiological analysis. Organic farmers supplied an average of 14 (±9.2 SD) samples each (range, 1 to 36 samples). The average number of samples that each conventional farmer supplied was 16 (±7.2 SD; range, 5 to 27 samples). The six produce types that made up 70% of the organic samples were tomatoes, leafy greens (kale, spinach, basil, amaranth, and Swiss chard), lettuce, green peppers, cabbage, and cucumbers (Table 1), and the remainder included broccoli, strawberries, apples, summer

TABLE 2. *Levels of coliform contamination in produce varieties from conventional and organic growers*

Produce variety	Coliform count (mean log MPN/g ± SD)[a]	
	Organic	Conventional
Tomato	2.3 ± 1.0 C	1.9 ± 1.4
Leafy greens	3.3 ± 1.8 AB	2.0 ± 0.1
Lettuce	4.0 ± 2.3 A	3.5 ± 2.1
Green pepper	2.0 ± 1.1 C	1.9 ± 1.8
Cabbage	2.6 ± 1.8 BC	1.6 ± 1.6
Cucumber	3.6 ± 1.5 AB	3.6 ± 1.9
Broccoli	3.0 ± 1.5 B	2.1 ± 0.8
Summer squash	3.9 ± 0.9	3.3 ± 0.6
Zucchini	2.6 ± 0.6	3.3 ± 1.7
Bok choi	3.0 ± 1.1	5.3 ± 2.6
Apple	2.7 ± 1.0	2.7 ± 1.0
Onion	3.6 ± 0.9	3.9 ± 2.2
Strawberry	2.7 ± 0.8	4.2 ± 1.4
Overall	2.9 ± 1.8 X	2.9 ± 1.8 X

[a] Data of produce types having different letters (A through C) were significantly different ($P < 0.05$) within the organic column. X indicates statistically significant differences between overall counts of organic and conventional produce. Statistical analysis was only done on the seven produce types that supplied >75% of the samples. No significance difference was found between the two columns for each produce type.

squash, bok choi, zucchini, cantaloupes, carrots, eggplant, raspberries, onions, beets, and kohlrabi. The most frequently collected conventional vegetables were tomatoes, cabbage, zucchini, green peppers, cucumbers, and onions. Conventional farmers provided the same fruits and vegetables as organic farmers, with the exception of raspberries, beets, and kohlrabi.

Coliform bacteria were detected in 92% of all the samples, and the overall average count in both organic and conventional produce was 2.9 log MPN g^{-1} (±1.8 SD) (Table 2). Organic leafy greens and broccoli had slightly higher mean counts of coliforms, but no differences were found when the coliform counts of lettuce, cabbage, tomatoes, green peppers, and cucumbers were compared between the two groups of farms. Organic lettuce and cucumber had more than 1.5 log MPN g^{-1} greater counts than tomatoes, cabbage, and green peppers, and these differences were statistically significant. When we determined the predominant coliform bacteria in 261 samples, *Enterobacter cloacae* and *Enterobacter sakazakii* were identified in 56 and 26%, respectively, of the highest dilution with growth in LST broth. *E. coli* was the predominant coliform in only five of those samples.

E. coli was isolated from 8% of all fruits and vegetables analyzed, and the average count of those samples that tested positive was 3.1 log MPN g^{-1} (±1.0 SD). The overall prevalence of *E. coli* in organic produce was approximately sixfold greater than in conventional fruits and vegetables, and this difference was statistically significant ($P < 0.05$) (Table 3). Organic lettuce had approximately 22.4% of samples positive for *E. coli,* and this level was significantly higher than that of leafy greens, cabbage, tomatoes, green peppers, cucumbers, and broccoli. When the prevalence of *E. coli* in the latter six types of organic produce was compared, no significant difference was found. No *E. coli* was detected in strawberries, apples, summer squash, raspberries, cantaloupe, carrots, beets, and kohlrabi.

The prevalence of *E. coli* on certified organic produce

TABLE 3. *Comparison of* Escherichia coli *prevalence in produce samples from certified and noncertified organic and conventional farms*

Produce variety	*Escherichia coli* prevalence % (no. of positive samples/no. of samples)			
	Organic			
	Certified	Noncertified	Total	Conventional
Tomato	14.3 (3/21)	2.8 (2/71)	5.4 (5/92) B	0 (0/16)
Leafy greens	0 (0/19)	13.8 (9/65)	10.7 (9/84) B	25.0 (1/4)
Lettuce	0 (0/10)	30.8 (12/39)	22.4 (12/49) A	16.7 (1/6)
Green pepper	16.7 (1/6)	8.1 (3/37)	9.3 (4/43) B	0 (0/12)
Cabbage	0 (0/9)	13.3 (4/30)	10.2 (4/39) B	0 (0/15)
Cucumber	0 (0/7)	8.3 (2/24)	6.4 (2/31) B	0 (0/9)
Broccoli	0 (0/9)	19.0 (4/21)	13.3 (4/30) B	0 (0/6)
Summer squash	0 (0/5)	0 (0/13)	0 (0/18)	0 (0/6)
Zucchini	25.0 (1/4)	0 (0/3)	14.3 (1/7)	0 (0/15)
Bok choi	0 (0/3)	16.7 (2/12)	13.3 (2/15)	0 (0/3)
Apple	0 (0/4)	0 (0/6)	0 (0/10)	0 (0/6)
Onion	0 (0/1)	37.5 (3/8)	33.3 (3/9)	0 (0/7)
Strawberry	0 (0/0)	0 (0/8)	0 (0/8)	0 (0/3)
Overall	4.3 (5/117) X	11.4 (41/359) Y	9.7 (46/476) Y	1.6 (2/129) X

[a] Data of produce types having different letters (A and B) were significantly different ($P < 0.05$) within the total organic column. X and Y indicate statistically significant differences between overall prevalence. Statistical analysis was only done on the seven produce types that supplied >75% of the organic samples.

TABLE 4. *Comparisons of* Escherichia coli *prevalence in samples of fruits and vegetables according to organic management practices*

Criteria of classification	Group	% positive (no. of samples)	Total no. of samples
Type of manure used by organic farms	Cattle manure	16 (25) A	157
	Others	6.6 (21) B	319
Age of manure used by organic farms	>1 year	1.0 (1) C	95
	½–1 year	25.3 (45) D	223
Time of application of compost/manure	Spring	5 (11) E	223
	Fall	10.7 (6) E	56

[a] Pairs of data having different letters were significantly different ($P < 0.05$).

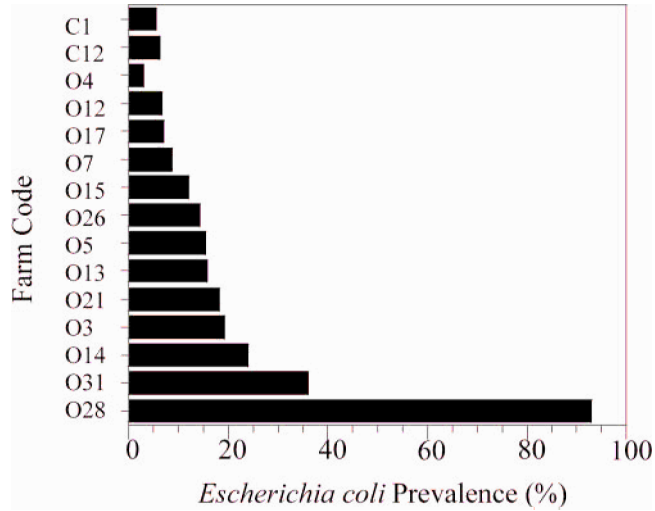

FIGURE 1. *Prevalence of* E. coli *in samples of individual farms. Farm codes starting with O and C are organic and conventional farms, respectively.*

(4.3%) appeared to be almost threefold higher (OR = 2.9) than that on conventional produce (1.6%), but this difference was not statistically significant ($P = 0.094$). The produce from certified organic farms was 2.6 times (OR = 2.8) less likely to have *E. coli* than vegetables from noncertified farms, and in this case, the difference was significant (Table 3). Among organic practices, the prevalence of *E. coli* was 2.4 times greater in produce grown on farms using cattle manure than in produce from farms using other types of manure (Table 4). Applying manure or compost in the spring or fall did not appear to be related with increased numbers of *E. coli* positive samples. However, organic samples from farms that used materials aged 6 to 12 months had 19 times greater (OR = 23.8) prevalence than those from farms that used material more than 1 year old.

A total of 15 farms had *E. coli*–positive samples, and two of those farms were conventional growers (Fig. 1). A total of 7 certified and 10 noncertified organic farms had no *E. coli*–positive sample. Among organic farms, almost all samples from farm O28 tested positive for *E. coli,* but less than 30% of samples from the remaining 12 farms were positive. The combined number of positive samples from the five organic farms (O28, O31, O14, O3, and O21) with higher prevalences accounted for 65% of all *E. coli* isolations from organic produce.

E. coli O157:H7 was not detected in any organic and conventional produce samples, but *Salmonella* was isolated from one organic lettuce and one organic green pepper collected at farms O14 and O17, respectively. Of interest, the samples contaminated with *Salmonella* tested negative for *E. coli.* None of the *E. coli* isolates tested positive for the presence of shiga toxin 1 and 2 genes.

DISCUSSION

The present study was intended to provide some assessment on the microbiological quality of organic fruits and vegetables. Based on largely unfounded reports in the media and the fact that organically grown crops often rely on animal wastes as fertilizers, these products are perceived to pose a greater risk for foodborne disease than conventional crops. However, there have been very few scientific reports that have conducted microbiological analysis of organic produce, and none of those have evaluated products at the farm level *(10, 20, 24).* By sampling during the preharvest stage, the level of microorganisms in the present study was only influenced by farm practices and the source of those bacteria was likely the farm environment.

For decades, *E. coli* has been used as the reference indicator for fecal contamination, and a number of surveys have reported its isolation from fresh fruits and vegetables *(14).* In a recent study that tested conventionally grown fresh produce at retail, only one sample tested positive for *E. coli* out of 50 samples that included alfalfa sprouts, broccoli, cauliflower, lettuce, celery, and mung bean sprouts *(34).* The percentage of *E. coli*–positive samples found in a survey of conventionally grown fresh vegetables in Japan (including cabbage, lettuce, onions, spinach, and celery) was 2% *(18).* These results are consistent with the prevalence of *E. coli* in conventionally grown produce (1.6%) that we found. However, in other reports, as many as 4 of 48 conventional spring mix samples had an average of 10^4 CFU g^{-1} *E. coli (9),* and the prevalence of this bacterium in ready-to-use lettuce was 25%, according to the results of a restaurant survey *(29).*

There are only three published microbiological surveys of organic fruits and vegetables, but all of these studies were conducted at the retail level. In an investigation in the United Kingdom that included 3,150 samples of ready-to-eat organic vegetables (including broccoli, cabbage, carrots, lettuce, mushrooms, tomato, cucumber, pepper, and others), the prevalence of *E. coli* was 1.5%, and most of the positive samples had less than 100 CFU g^{-1} *(24). E. coli* was not detected in 86 organic vegetable samples, but *Aeromonas* species were found in 34% of samples *(20).* The prevalence of *E. coli* in 48 samples of organic spring mix reported by Doyle *(9)* was significantly greater: as many as 16.7% positive samples were detected, and a markedly large average count of 10^6 CFU g^{-1} was reported. The overall *E. coli* prevalence in organic produce calculated in the present study appeared to be intermediate between these studies,

although the prevalence in lettuce was even higher than in the Doyle study (Table 3).

Among fresh fruits and vegetables, lettuce appears to be more susceptible to bacterial contamination. Not only have a number of outbreaks caused by *E. coli* O157:H7 *(33)* been linked to the consumption of lettuce, but recent evidence suggests that foodborne pathogens can be internalized into lettuce leaves *(28).* That report provided evidence that O157:H7 could be transmitted from contaminated manure and irrigation water applied to the soil into the subsurface tissues of lettuce leaves. In the present study, all of the lettuce samples were negative for *E. coli* O157:H7, but the prevalence of total *E. coli* was significantly higher in both organic and conventional lettuce than in any other produce varieties (Table 3).

Animal and plant wastes are the major sources of fertility for organic production. According to organic regulations, animal waste should be composted to reach internal temperatures of 55 to 77°C (composted manure), but untreated manure may be applied at least 90 to 120 days (aged manure) before harvesting the product, depending on whether it could come in contact with soil *(1).* In a recent nationwide survey of organic producers, 43% of the respondents indicated that they never used untreated manure, whereas 22% stated that they applied untreated manure regularly. In contrast, waste plant materials (green manures) and composted materials were used regularly by 72 and 59%, respectively, of the respondents *(37).* In the present study, all of the participating organic farmers reported using aged or composted manure, but it should be noted that the outlier farm O28, which had a 90% prevalence of *E. coli* contamination, spread untreated manure even during the harvest season.

The present study found a marked difference in the prevalence of *E. coli* between the samples from certified and noncertified organic farms (Table 3). Among organic farms, the percentage of those that had at least one positive sample for *E. coli* were 12 and 59% for certified and noncertified growers, respectively. Considering that the fertilization practices of the certified organic farms would fulfill the USDA requirements for manure fertilizer, this significant difference may reflect the importance of certification as a potential means to ensure minimum fecal contamination of fruits and vegetables. Ours is the first study that suggests a potential association between organic certification and reduced *E. coli* prevalence. Further research is recommended to confirm this finding.

E. coli is a natural inhabitant of the gastrointestinal tract of most warm-blooded animals, and it can be easily isolated from the feces of most livestock species *(8).* However, among the various types of manure, the use of cattle manure alone or in combination with other animal manures appeared to be related to an increased number of *E. coli*–positive samples as compared to other types of manure. This finding is intriguing because there is very little published evidence that may support the idea that cattle shed more *E. coli* than other animals. However, the absence of *E. coli* O157:H7 in any of the samples from farms that used cattle manure did not support this relationship. On the basis of the observation that approximately 50% of the *E. coli*–positive samples originated from farms that used cattle manure and the studies that have reported a relatively high prevalence of Shiga toxin–producing *E. coli* in cattle feces *(21),* we screened all *E. coli* isolates for the presence of shiga toxin genes. None of them, however, carried either *stx1* or *stx2*.

Fresh vegetables have been identified as the vehicle for *E. coli* O157:H7 infection in approximately 19 outbreaks in the United States *(23, 26).* Despite the fact that epidemiological investigations have found very strong evidence that has linked most of these cases to the implicated produce, very few have been successful in isolating O157:H7 from the products *(12).* A number of surveys have attempted to detect *E. coli* O157:H7 in fresh fruits and vegetables. In a study that included 3,200 organic retail vegetables, no O157:H7-positive sample was detected, and in another survey of 890 fruits and vegetables, this pathogen could not be found either *(17, 24).* Consistent with these previous studies, this investigation did not find any evidence of O157:H7 contamination during the preharvest stage.

Multiple studies have shown that *Salmonella* can be isolated from fresh produce, and the prevalence of *Salmonella* in healthy whole fresh vegetables can be as high as 8% *(4, 9).* In recent surveys of fruits and vegetables in the retail markets of Norway and the United States, however, no *Salmonella*-positive samples were found *(17, 34).* Similarly, a variety of organic fresh produce was reported to be free of *Salmonella* in two studies conducted in the United Kingdom *(20, 24).* In a study that included 39 samples of organic alfalfa sprouts, 3 samples tested positive for *Salmonella (9).* The prevalence of *Salmonella* in organic fruits and vegetables found in the present study (which did not include alfalfa sprouts) was only 0.4%. Based on the absence of *E. coli* O157:H7 and the very low *Salmonella* prevalence, the assertion that organic produce has greater pathogen contamination does not seem to be supported.

The present study was designed to provide an initial estimation on the microbiological quality of organic fruits and vegetables based on collection of samples directly from farms. Some of the characteristic features of the study were the random collection of samples, the variety of fruits and vegetables, and the diversity of farms. However, our results could have been influenced by the unbalanced numbers of samples among produce varieties, the potential effects of weather and geographic location, and the natural fluctuations that may occur in microbial populations. The populations of epiphytic bacteria (organisms that can grow on the intact surface of plants) can be changed by relative humidity, rain, temperature, and ultraviolet light *(32).* Resident epiphytes such as *Pseudomonas syringae* and *Erwinia* species are capable of growing and tolerating relative adverse conditions *(22).* Little is known about the ability of *E. coli* and *Salmonella* as transient epiphytes, but it should be expected that their populations could be markedly affected by conditions such as sunlight and dryness. Additional work is needed to generate a more comprehensive data set that would address the influence of those variables on bacterial populations and the potential for foodborne disease risk.

The results of the present study do not support allegations that organic produce poses a substantially greater risk of pathogen contamination than does conventional produce. However, the observation that the prevalence of *E. coli* was significantly higher in organic produce supports the idea that organic produce is more susceptible to fecal contamination. Our results also suggest that organic certification might further reduce the likelihood of fecal contamination in organic produce. Further comparative analyses of organic and conventional produce at the farm level, coupled with detailed documentation of production practices, are needed to corroborate these results.

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REFERENCES

1. Agricultural Margeting Service. 2000. National organic program. Final rule. 7 CFR part 205. U.S. Department of Agriculture, Washington, D.C.
2. Andrews, W. H., and T. S. Hammack. 1998. *Salmonella,* p. 5.01–5.20. In U.S. FDA bacteriological analytical manual, 8th. ed. AOAC International, Gaithersburg, Md.
3. Avery, D. T. 2002. Hidden dangers in organic food. Hudson Institute. Available at: http://www.cgfi.org/materials/articles/2002/jun_25_02.htm.
4. Beuchat, L. R. 1995. Pathogenic microorganisms associated with fresh produce. *J. Food Prot.* 59:204–216.
5. Bland, J. M., and D. G. Altman. 2000. The odds ratio. *BMJ* 320:1468.
6. Bolton, J. F., L. Crozier, and J. K. Williamson. 1995. Optimization of methods for the isolation of *Escherichia coli* O157:H7 from beefburgers. *PHLS Microbiol. Digest.* 12:67–70.
7. Bourn, D., and J. Presscott. 2002. A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. *Crit. Rev. Food Sci. Nutr.* 42:1–34.
8. Dombek, P. E., L. K. Johnson, S. T. Zimmerly, and M. J. Sadowsky. 2000. Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources. *Appl. Environ. Microbiol.* 66:2572–2577.
9. Doyle, M. P. 2000. Reducing foodborne disease: what are the priorities? *Nutrition* 16:647–694.
10. Doyle, M. P. 2001. Keeping food borne pathogens down the farm. USDA Food Safety and Inspection Service. Available at: http://www.fsis.usda.gov/OPPDE/animalprod/Presentations/KFPPF%20Aug%2001/.
11. Economic Research Service, U.S. Department of Agriculture. 2001. U.S. organic agriculture. Available at: http://www.ers.usda.gov/emphases/harmony/issues/organic/organic.html.
12. Hilborn, E. D., J. H. Mermin, P. A. Mshar, J. L. Hadler, A. Voetsch, C. Wojtkunski, M. Swartz, R. Mshar, M.-A. Lambert-Fair, J. A. Farrar, M. K. Glynn, and L. Slutsker. 1999. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. *Arch. Intern. Med.* 159:1758–1764.
13. Hitchins, W. D., P. Feng, W. D. Watkins, S. R. Rippey, and L. A. Chandler. 1995. *Escherichia coli* and the coliform bacteria, p. 4.01–4.29. *In* FDA bacteriological analytical manual, 8th. ed. AOAC International, Gaithersburg, Md.
14. Jay, J. M. 2000. Modern food microbiology, 6th. ed. Aspen Publishers, Gaithersburg, Md.
15. Jiang, J., C. Larkin, M. Steele, C. Poppe, and J. A. Odomeru. 1998. Evaluation of Universal pre-enrichment broth for the recovery of foodborne pathogens from milk and cheese. *J. Dairy Sci.* 81:2798–2803.
16. Jiang, X., J. Morgan, and M. P. Doyle. 2003. Fate of *Escherichia coli* O157:H7 during composting of bovine manure in a laboratory-scale bioreactor. *J. Food Prot.* 66:25–30.
17. Johannessen, G. S., S. Loncarevic, and H. Kruse. 2000. Bacteriological analysis of fresh produce in Norway. *Int. J. Food Microbiol.* 77:199–204.
18. Kaneko, K.-I., H. Hayashidani, Y. Ohtomo, J. Kosuge, M. Kato, K. Takahashi, Y. Shiraki, and M. Ogawa. 1999. Bacterial contamination of ready-to-eat foods and fresh products in retail shops and food factories. *J. Food Prot.* 62:644–649.
19. Lung, A. J., C.-M. Lin, J. M. Kim, M. R. Marshall, R. Nordstedt, N. P. Thomson, and C. I. Wei. 2000. Destruction of *Escherichia coli* O157:H7 and *Salmonella entritidis* in cow manure composting. *J. Food Prot.* 64:1309–1314.
20. McMahon, M. A. S., and I. G. Wilson. 2001. The occurrence of enteric pathogens and *Aeromonas* species in organic vegetables. *Int. J. Food Microbiol.* 70:155–162.
21. Midgley, J., N. Fegan, and P. Desmarchelier. 1999. Dynamics of shiga toxin–producing *Escherichia coli* (STEC) in feedlot cattle. *Lett. Appl. Microbiol.* 29:85–89.
22. O'Brian, R. D., and S. E. Lindow. 1988. Effect of plant species and environmental conditions on epiphytic population sizes of *Pseudomonas syringae* and other bacteria. *Phytopathology* 79:619–627.
23. Olsen, S. J., L. C. MacKinon, J. S. Goulding, and L. Slutsker. 2000. Surveillance for foodborne disease outbreaks—United States, 1993–1997. *Morb. Mortal. Wkly. Rep.* 49:1–51.
24. Sagoo, S. K., C. L. Little, and R. T. Mitchell. 2001. The microbiological examination of ready-to-eat organic vegetables from retail establishments in the United Kingdom. *Lett. Appl. Microbiol.* 33:434–439.
25. Sloan, A. E. 2002. The natural & organic foods marketplace. *Food Technol.* 56:27–37.
26. Smith DeWaal, C., K. Barlow, L. Alderton, and M. F. Jacobson. 2002. Outbreak alert! Center for Science in the Public Interest. Available at: http://www.cspinet.org/reports/outbreak_report.pdf.
27. Snedecor, G. W., and W. G. Cochran. 1989. Statistical methods. Iowa State University, Ames.
28. Solomon, E. B., S. Yaron, and K. R. Mathews. 2002. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Appl. Environ. Microbiol.* 68:397–400.
29. Soriano, J. M., H. Rico, J. C. Molto, and M. J. 2000. Assessment of the microbiological quality and wash treatments of lettuce served in University restaurants. *Int. J. Food Microbiol.* 58:123–128.
30. Stossel, J. 4 February 2000. The food you eat, 20/20, ABC News.
31. Strockbine, N., and O. Olsvik. 1993. PCR detection of heat-stable, heat labile, and shiga-like genes in *Escherichia coli*, p. 271–276. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology, principles and applications. ASM Press, Washington, D.C.
32. Suslow, T. 2002. Production practices affecting the potential for persistent contamination of plants by microbial foodborne pathogens, p. 241–256. *In* S. E. Lindow, E. I. Hecht-Poinar, and V. J. Elliott (ed.), Phyllosphere microbiology. APS Press, St. Paul, Minn.
33. Tauxe, R., H. Kruse, C. Hedberg, M. Potter, J. Madden, and K. Wachsmuth. 1997. Microbial hazards and emerging issues associated with produce a preliminary report to the national advisory committee on microbiological criteria for foods. *J. Food Prot.* 60:1400–1408.
34. Thunberg, R. L., T. T. Tran, R. W. Bennett, R. N. Matthews, and N. Belay. 2002. Microbial evaluation of selected fresh produce obtained at retail markets. *J. Food Prot.* 65:677–682.
35. Tschape, H., R. Prager, W. Streckel, A. Fruth, and E. Tietze. 1995. Verotoxinogenic *Citrobacter freundii* associated with severe gastroenteritis and cases of haemolytic uraemic syndrome in a nursery school: green butter as the infection source. *Epidemiol. Infect.* 114:441–450.
36. Turner, C. 2002. The thermal inactivation of *E. coli* in straw and pig manure. *Biores. Technol.* 84:57–61.
37. Walz, E. 1999. Third biennial national organic farmers' survey. Organic Farming Research Foundation, Santa Cruz, Calif.