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Research Note

Microbial Survey of Selected Ontario-Grown Fresh Fruits and Vegetables

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ABSTRACT

Recent produce-related outbreaks have been receiving heightened media coverage, which has increased public concern toward the safety of fresh fruits and vegetables. In response, the microbial contamination of Ontario-grown fresh fruits and vegetables was evaluated by the Ontario Ministry of Agriculture, Food and Rural Affairs during the summer of 2004. Prior to this survey, information specific to the microbial contamination of Ontario-produced fruits and vegetables was limited. This nonregulatory survey had two objectives: (i) to obtain a general microbiological profile of selected fruits and vegetables produced in Ontario and (ii) to use the information and knowledge gained from this survey to direct and support future on-farm food safety research and food safety programs to manage potential risks. In all, 1,183 samples, including muskmelon (151), scallions and green onions (173), leaf lettuce (263), organic leaf lettuce (112), head lettuce (155), parsley (127), cilantro (61), and fresh market tomatoes (141), were collected and analyzed. Samples were analyzed for *Salmonella, Shigella,* and generic *E. coli.* Enrichment cultures positive for *E. coli* were further assessed for verotoxigenicity. One sample each of Roma tomato and organic leaf lettuce were positive for *Salmonella,* with no samples yielding *Shigella* or verotoxigenic *E. coli.* The *E. coli* prevalence was highest in parsley (13.4%), followed by organic leaf lettuce (11.6%), leaf lettuce (6.5%), scallions (6.4%), cilantro (4.9%), muskmelon (1.3%), head lettuce (0%), and fresh market tomatoes (0%). These findings, in combination with foodborne illness data, will help target those commodities that require more focused risk mitigation efforts.

Microbial foodborne illness is the largest class of emerging infectious disease in Canada *(1).* Until recently, fresh fruits and vegetables were considered safe relative to foods of animal origin *(10, 11, 19).* Recently, there has been increased attention on the potential for fresh fruits and vegetables to be a vector for illness *(3, 7, 9, 10, 19).* Increased focus on produce as a food safety risk can be explained by several large outbreaks. These include a 1996 to 1997 outbreak involving over 1,000 cases of *Cyclospora* in the United States that were attributed to Guatemalan raspberries *(6, 16)*, the 2003 hepatitis A outbreak in the United States with over 650 cases traced to Mexican scallions (green onions) *(5, 17)*, a 2005 Ontario *Salmonella* outbreak in which over 600 cases of salmonellosis were attributed to the consumption of mung bean sprouts *(18)*, and a recent outbreak in the United States and Canada involving spinach in which approximately 200 individuals were infected with *E. coli* O157:H7 *(21).*

Assessing the relative risk of domestically produced produce is difficult because most information on the health impacts from these commodities originates from the United States. This information includes microbial surveys conducted by the U.S. Food and Drug Administration (FDA) on domestic fresh produce in 2003 *(22)* and the U.S. Department of Agriculture (USDA) Microbiological Data Program (MDP) conducted from 2002 to 2006 *(20).*

Ontario's climate, culture, and production practices are not necessarily reflected by the United States. Therefore, Ontario requires specific food safety information that includes the fecal indicator *E. coli,* in addition to pathogen prevalence and foodborne illness epidemiological information, to make informed decisions regarding the direction of future intervention, inspection, food safety programs, and research efforts.

To address this situation, the Ontario Ministry of Agriculture, Food and Rural Affairs conducted a survey to obtain general microbiological profiles on selected Ontario-grown fresh fruits and vegetables. The objective of this study was to obtain information and knowledge to direct and support future on-farm food safety research and food safety programs to manage potential risks.

MATERIALS AND METHODS

Study design. Retail distribution centers (DCs) and farmers' markets (FMs) were chosen as sample collection points, with organic wholesale locations or organic farms (ORGs) chosen secondarily when organic lettuce was not available at DCs or FMs. The ratio of planned samples collected from DCs relative to FMs was 9:1 based on a rough approximation of the volume of consumer sales compared with FMs.

Sampling was restricted to DCs, FMs, and ORGs in south-

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western and south-central Ontario that were open during regular weekday business hours. Together, these regions represent the greatest percentage of Ontario-grown fruits and vegetables distributed. This geographical area for sample pick-up was also conducive with the short Ontario growing season and budget constraints. A sampling framework for 12 DCs, 17 FMs, and 5 ORGs was developed for a planned sample collection period of 8 weeks, with an additional 2-week contingency period, if necessary.

Sample collection. Two individuals trained in aseptic sampling and random sampling methods carried out the planned sampling. These individuals also received technical and scientific information specific to the commodities being surveyed and were familiar with both the objectives of the study and the sample submission forms. The Canadian Food Inspection Agency and Ontario Ministry of Agriculture, Food and Rural Affairs staff conducted all training collaboratively.

All samples were collected in 2004 during the August to September Ontario harvest season and were shipped in refrigerated coolers within 24 h of collection to the Laboratory Services Division, University of Guelph, for microbiological analysis. Sample collection was randomized, with each weekly schedule indicating the sample type, number of samples to be collected, and location where samples were to be obtained. Backup locations were also randomly generated if certain sample types were not available at the originally specified locations.

Types of produce evaluated. A total of 1,183 samples were evaluated, including Ontario-grown muskmelon (151), scallions and green onions (173), leaf lettuce (263), organic leaf lettuce (112), head lettuce (155), parsley (127), cilantro (61), and fresh market tomatoes (141). These products were selected on the basis of the outbreak data, the production and handling methods, whether the product is consumed fresh, the amount that is typically consumed, the structural characteristics, and the need for additional food safety–related information. The number of samples allocated to each commodity was based on perceived health risk and statistical considerations while also considering financial and seasonal constraints.

Microbiological sampling. Sterile scalpels and forceps were used to cut and transfer 25-g samples of the fruits and vegetables into sterile stomacher bags. For muskmelon, both the inside and outside portions of the melon were sampled, because the internal portion could potentially contain pathogens. All samples were diluted 1:10 in 225 ml of buffered peptone water (1% buffered peptone, pH 7.2), homogenized in a stomacher for 2 min, and then examined for the indicator organisms and pathogens as described below.

Microbiological analyses. All samples were analyzed for *Salmonella, Shigella,* and *E. coli,* and those samples positive for *E. coli* were further tested for the presence of verocytotoxigenic *E. coli.* The pH of the homogenized sample was measured prior to culture with a Whatman pH indicator strip and adjusted to pH 6 to 7 with 1 M NaOH or 1 M HCl if necessary.

Detection and enumeration of *E. coli* were performed by the Petrifilm method *(12).* Three dilutions were prepared to obtain minimum and maximum detection limits of 5×10^5 to 1.5×10^5 CFU/g. To detect the presence of verocytotoxigenic *E. coli* in *E. coli*–positive samples, a sweep of multiple blue and nonblue colonies from the Petrifilm plates was inoculated into brain heart infusion broth and incubated for 24 h at 35 ± 2°C. The broth culture was centrifuged at 8,000 × *g* for 2 to 3 min, and 50 μl of the supernatant was tested for presence of *E. coli* verotoxin by the Verocell Assay, described in the MFLP-89 *(14).* Enrichment cultures positive for *E. coli* were further tested for the presence of verocytotoxigenic *E. coli* by a vero cell assay and culture method based on the Health Canada Compendium of Analytical Methods, MFLP-89 *(14). Salmonella* and *Shigella* were detected by the enrichment protocols described in the Health Canada Compendium of Analytical Methods MFHPB-20 *(13)* and MFLP-25 *(15),* respectively. Identification of suspect *Salmonella* colonies was performed with the automated Microscan Walkaway 40 microbial identification system (Dade Behring Inc., West Sacramento, Calif.). Serotyping of *Salmonella* isolates was performed at the Health Canada *Salmonella* Reference Center, Guelph, Ontario.

Statistical analysis. Data were analyzed by SAS for Windows, version 9.1 (SAS Institute Inc., Cary, N.C.). Prevalence analysis was performed by the PROC FREQ procedure, with all laboratory results below the limit of detection (<5 CFU/g) treated as ''negative'' findings and all laboratory results at 5 CFU/g or above treated as ''positive'' findings in calculations of prevalence estimates. Chi-square analysis was used to test for significant differences between prevalence estimates for each commodity ($\alpha = 0.05$).

RESULTS

Pathogen prevalence. Of the 1,183 samples of fresh fruits and vegetables analyzed, only two samples—one each of Roma tomato and organic leaf lettuce—yielded *Salmonella,* with both isolates identified as *Salmonella* Schwarzengrund. These two samples came from different DC locations and were analyzed separately. Statistical analysis did not detect any differences between the pathogen prevalence rates on fresh market tomatoes or organic leaf lettuce compared with any of the other commodities evaluated, with all *P* values >0.10). No samples were positive for *Shigella* or verotoxigenic *E. coli.*

***E. coli* prevalence.** *E. coli* prevalence comparisons among commodity types resulted in a number of statistically significant differences ($P < 0.05$). The prevalence of *E. coli* in parsley was significantly higher than all other commodities, except for organic leaf lettuce (similar prevalence) and cilantro (because of the low number of samples). In addition, scallions and both organic and conventional leaf lettuce each had a significantly higher prevalence of *E. coli* than did muskmelon, tomatoes, and head lettuce, all of which had prevalence rates of 2% or less. Prevalence of *E. coli* in cilantro was significantly higher than in tomatoes and head lettuce (Table 1).

When detected, *E. coli* was typically present at levels of <1 log CFU/g (Fig. 1); however, *E. coli* did exceed 3 log CFU/g in some samples of parsley, cilantro, and scallions.

Location type. Eighty-two percent of the samples were obtained from DCs, 15% from FMs, and 2% from ORGs. The overall prevalence rates of *E. coli* did not significantly differ ($P = 0.76$) by location type: 5.5% at DCs, 4.4% at FMs, and 3.6% at ORGs.

DISCUSSION

Low prevalence rates were found for the pathogens examined in this survey. These findings are similar to those from other surveys, including the FDA survey of domestic

TABLE 1. E. coli *prevalence and range of* E. coli *recovered for all commodities evaluated*

Commodity type	No. of samples (% positive)[a]	Range (CFU/g)	Commodities that are significantly different ($P < 0.05$)[b]
All commodities	1,183 (5.3)	<5–16,000	
All lettuce types	530 (5.7)	<5–290	
Head	155 (0)	<5	P, OL, LL, S, C
Leaf—conventional	263 (6.5)	<5–260	P, M, HL, T
Organic leaf	112 (11.6)	<5–290	M, HL, T
Cilantro	61 (4.9)	<5–7,600	HL, T
Parsley	127 (13.4)	<5–16,000	LL, S, M, HL, T
Scallions	173 (6.4)	<5–7,400	P, M, HL, T
Muskmelon	151 (1.3)	<5–5	P, OL, LL, S, C
Tomatoes	141 (0)	<5	P, OL, LL, S, C

[a] Percent positive = percentage of samples that contained ≥5 CFU/g.

[b] P, parsley; OL, organic leaf lettuce; LL, leaf lettuce; S, scallions; C, cilantro; M, muskmelon; HL, head lettuce; T, tomato. Significantly different ($P < 0.05$) as tested by a chi-square.

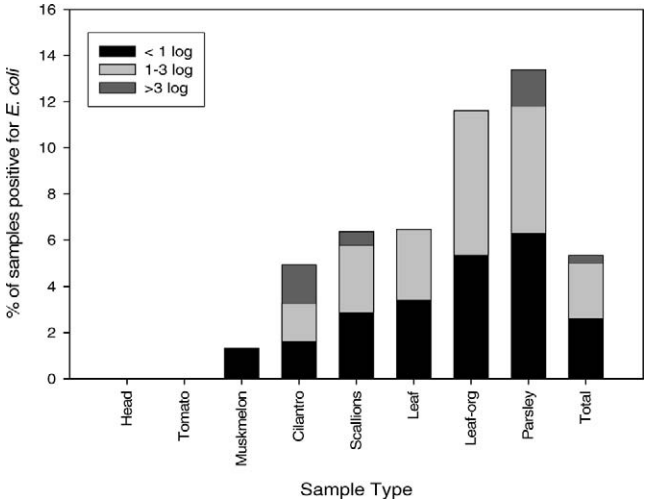

FIGURE 1. *Prevalence and levels of* E. coli.

fresh produce and the USDA MDP, in which 1.10 and 0.67% of the samples contained pathogens, respectively *(20, 22).* However, these results likely underestimate the relative concern toward produce as a vector for disease, as produce is increasingly being implicated as a vehicle for foodborne outbreaks *(3).* The sporadic nature of microbial contamination of produce provides an explanation for the low pathogen prevalence found in microbial surveillance studies, as this makes it difficult to identify contaminated products.

To obtain better estimates of the true pathogen prevalence rates on fresh fruits and vegetables, a greater number of samples would be required. Because of financial constraints and the short duration of the Ontario growing season, it is difficult to obtain the numbers of samples necessary to achieve more precise pathogen prevalence estimates.

E. coli was chosen as the best available indicator for fecal contamination. Fecal material has the potential to harbor enteric pathogens; as a result, *E. coli* contamination on fresh produce should be kept at a minimum. In this study, cilantro, parsley, leafy greens, and scallions were more frequently contaminated with *E. coli* and at higher levels than the other products, reflecting recent outbreaks that have occurred in North America *(5, 11).* These commodities have large surface areas, are more frequently handled by employees, require irrigation because of their high transpiration rates, and are grown close to the ground. They also have a relatively short growing period, which reduces their exposure to environmental stress, enabling more pathogens to survive, if present, and potentially increasing the likelihood for involvement in foodborne outbreaks.

Fresh market tomatoes and muskmelon had a lower proportion of *E. coli*–positive samples. The authors anticipated higher *E. coli* counts on fresh market tomatoes and muskmelon, because these products have been linked to outbreaks of salmonellosis in North America *(7, 8),* and both *E. coli* and *Salmonella* are transmitted by fecal contamination. However, the USDA MDP also reported similar findings for *E. coli,* with tomatoes having the lowest prevalence for *E. coli* (6.12%), followed by cantaloupe (14.68%), lettuce (19.22%), green onions (23.83%), and sprouts (40.48%) *(20).* The FDA domestic survey did not report generic *E. coli* results.

In addition to a low *E. coli* prevalence on commodities historically associated with *Salmonella* contamination in both the Ontario survey and the USDA MDP, samples positive for *Salmonella* in Ontario had nondetectable levels of *E. coli.* These results question the validity of using *E. coli* as an indicator of *Salmonella* contamination. Consequently, the use of generic *E. coli* results in combination with other food safety information, such as pathogen prevalence and foodborne illness epidemiologic information, may be more appropriate in determining what foods of plant origin require focused risk mitigation efforts.

The results of both the Ontario survey and the USDA MDP survey must be considered in relative terms, as both studies used different methods of analysis. There are a number of different approaches to preparing samples for analysis *(2, 4).* Use of a single sampling protocol to accurately determine both presence and numbers of bacterial pathogens on all types of produce is unlikely, because of differences in commodity size, shape, and morphological features. The use of different procedures among samples, however, raises challenges in how to report and compare the data. It was decided for the purposes of this study to use the standard procedure outlined in the official methods by Canadian Food Inspection Agency–Health Canada for all samples. The authors realize that this single protocol may not have achieved maximum recovery of all target organisms, including generic *E. coli* from all samples evaluated.

The combined information from foodborne outbreaks and *E. coli* prevalence surveys, regardless of the low pathogen prevalence found in the Ontario, USDA MDP, and FDA domestic surveys *(20, 22),* suggests that produce can harbor fecal contamination and be a vector for disease.

Therefore, the authors suggest that producers implement proper Good Agricultural Practices to reduce the likelihood of pathogen contamination from the production environment.

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