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Abstract

To focus future good agricultural practices (GAP) educational efforts, we surveyed leafy greens growers about their farming practices and measured microbial load on greens. Most survey respondents used potable water without added sanitizer for washing. Pathogenic bacterial levels were undetectable or low on farm and farmers' market samples. Coliform counts were in the normal range and were similar across farms and treatments. The microbial food safety risk from locally grown leafy greens appeared to be low. Therefore, future outreach efforts can focus on worker hygiene and cleanliness of farming operations, making GAP training less onerous for growers.

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Introduction

According to the Centers for Disease Control and Prevention (2015), about 48 million Americans get sick each year due to food-borne illnesses. Since 2005, there have been 48 outbreaks due to *Escherichia coli* and *Salmonella* linked to leafy greens and lettuce in the United States (Centers for Disease Control and Prevention, 2015). As a consequence of outbreaks due to ingestion of fresh produce, the Food Safety Modernization Act (FSMA), which establishes science-based standards for the safe production and handling of produce for human consumption, was enacted (Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption, 2015). FSMA provides qualified exemptions for farms that sell the majority of their product directly to consumers or establishments within a 275-mi radius of production and have annual sales averaging less than $500,000.

These exemptions would apply to a majority of Minnesota farms selling produce, as 92% of Minnesota vegetable growers responding to a survey (Hultberg, Schermann, & Tong, 2012) stated that they farm 30 ac or less, and 75% market through farmers' markets. Farming small acreages, they are unlikely to complete food safety plans unless required to do so (Nayak, Tobin, Thompson, Radhakrishna, & LaBorde, 2015).

The University of Minnesota has provided education to produce farmers on food safety risks and good agricultural practices (GAP) for over 20 years. To determine the current level of food safety risk from locally grown leafy greens, we surveyed lettuce growers on their current GAP usage and measured microbial contamination on leafy greens from farms and farmers' markets. Although leafy greens are a minor component of Minnesota farm sales (Hultberg et al., 2012), we focused on leafy greens because they are generally eaten raw by Americans and have been the source of past food-borne outbreaks. Results from the study are useful for designing GAP outreach efforts.

Methods

Survey of Lettuce Growers

Leafy greens growers attending the annual meeting of the Minnesota Fruit and Vegetable Growers Association, the annual Immigrant and Minority Farmers Conference (IMFC), and farm food safety workshops were asked to respond to a short, written survey. The survey was designed to collect information about grower practices related to fertilizers, wash/rinse water, drying, and storage. At the IMFC, questions were interpreted by Hmong-speaking staff. The final survey question addressed whether respondents would be willing to have a member of the research team come to their farms to observe their handling practices. The University of Minnesota Institutional Review Board granted approval of the study with exempt status and determined that there was minimal risk to participants. Respondents were given a $20 gift card for participating.

Sampling and Microbiological Analyses

Eight organic farmers, half of whom used sanitizer and half of whom did not, agreed to collaborate on the project in 2014 and allowed us to sample one to th`ree times during the growing season. Three of the farmers agreed to wash samples with and without sanitizer after the first sampling date. Six farmers who used conventional methods or animal manure for fertility collaborated in 2015. In addition, samples were obtained from seven farmers' market stalls.

Samples (3–5 replicates) of leafy greens were harvested directly from the field, after washing, and from coolers (if available). Field samples were collected by researchers wearing gloves and wiping knives with ethanol between samples. Farmers washed samples according to their usual practice, and researchers bagged washed samples. All samples were immediately placed into sterile Whirl-Pak (Nasco, Fort Atkinson, WI) bags and transported in coolers with ice to the laboratory and then stored at 4°C. All samples were processed within 48 hr for the presence of *Salmonella* spp. and *Listeria* spp. and levels of *E. coli* and coliform bacteria.

For *E. coli* and coliform analyses, 11-g duplicates were prepared from samples and diluted tenfold with physiological saline. Specimens were stomached for 1 min and diluted in series. One milliliter of each dilution was plated on 3M *E. coli*/coliform count plate Petrifilm (3M Microbiology Products, Saint Paul, MN) and incubated at 37°C. The total numbers of blue (*E. coli*) and colorless (coliforms) colonies were counted after 48 hr. Average coliform counts were calculated and subjected to analysis of variance and Tukey's honestly significant difference using R software (R Core Team, 2014) to determine the extent of differences among treatments. *Salmonella* and *Listeria* analyses were done according to the Bacteriological Analytical Manual (Andrews, Jacobson, & Hammack, 2015; Hitchins, Jinneman, & Chen, 2016).

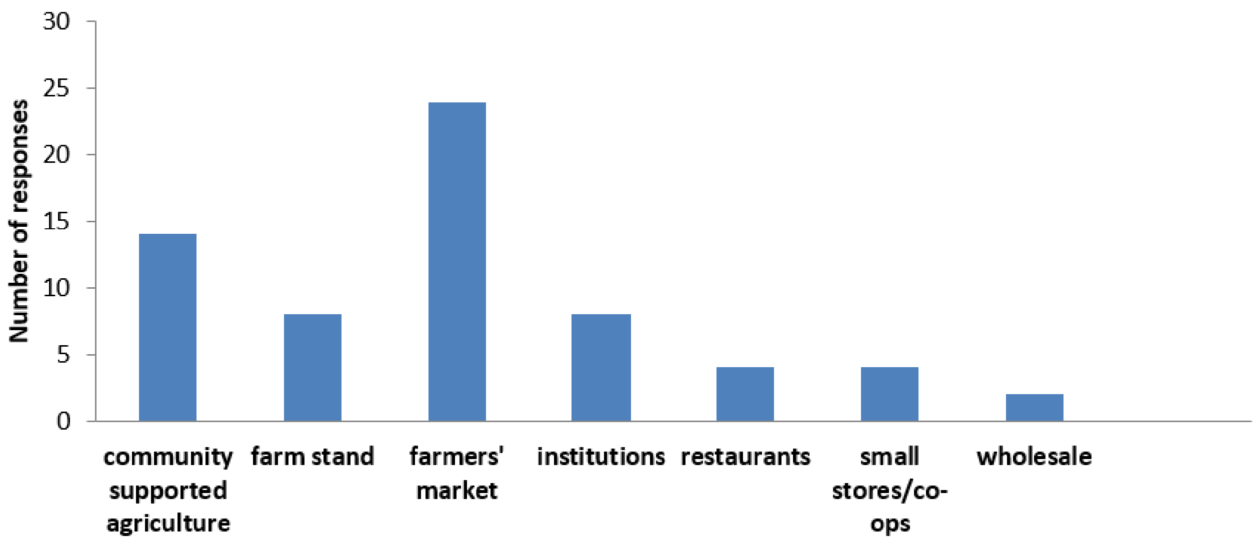
Results

Survey of Lettuce Growers

Thirty-nine farmers answered our survey about leafy greens production practices. We do not know how representative these respondents are of all Minnesota farms growing and selling leafy greens as there is no available census of Minnesota vegetable farmers who raise leafy greens for sale. All farms in the study except one were located in southern and central Minnesota. Fifty-nine percent of respondents were immigrant or minority farmers, and 41% were White nonimmigrants. The most highly used marketing outlet was farmers' markets (38%), followed by community-supported agriculture systems (22%), farm stands (12.5%), institutions such as schools and hospitals (12.5%), restaurants (6%), small stores or co-ops (6%), and wholesalers (3%) (Figure 1). Respondents chose as many outlets as applied to their operations.

Figure 1.

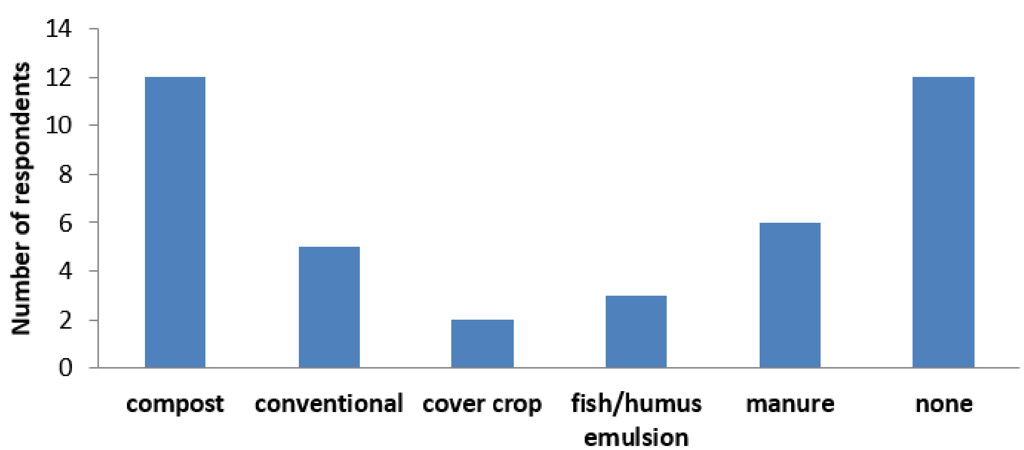
Marketing Outlets Used by Respondents to Minnesota Lettuce Grower Survey



Thirty-two percent of respondents did not fertilize leafy greens, 31% used composted animal or green manure as a soil amendment, 16% used raw manure, and 13% used conventional fertilizers (Figure 2).

Figure 2.

Soil Amendments Used by Respondents to Minnesota Lettuce Grower Survey



Almost all respondents (98%) washed leafy greens. Of those, 21% added sanitizer to the wash water, 74% did not use sanitizer, and 5% did not respond to the associated question. Only one of eight respondents who added sanitizer to wash water determined whether the correct amount of sanitizer was being used. The percentages of respondents using well water, municipal water, or rainwater for washing were 44%, 26%, and 3%, respectively; 27% did not answer the associated question. Respondents used multiple methods, including dunking (78% of respondents), spraying (23% of respondents), or both methods (5% of respondents). Wash water was most often changed "when dirty" (59%). Otherwise, wash water was changed once or twice a day (13%), or after every batch of greens (10%); 18% did not respond to the associated question. Wash water temperature was not monitored by 83% of respondents.

Most respondents (77%) dried greens. As with washing, multiple drying methods were used by respondents, including spin-drying (55%), drip-drying (39%), and drying with towels (6%). Similarly, 67% of respondents stored greens in coolers (26% did not store greens, and 7% did not answer the associated question), and 75% of those respondents who stored greens in coolers checked cooler temperatures.

The high percentage of respondents using potable water suggests low food risk from wash water, but the low percentage of sanitizer use and testing was concerning. We surmise that most growers perceive that sanitizers are unnecessary, an added expense, and cumbersome to use.

Microbial Contamination of Leafy Greens

The microbial sampling was performed with a subset of surveyed growers. In 2014, no *Salmonella* or *E. coli* was detected in any samples. Two samples were presumptive positive for *Listeria* spp., but we did not determine whether they were *L. monocytogenes*, the species that causes listeriosis. The levels of coliforms were all less than 4 $\log_{10}$ colony forming units (CFU) per gram of lettuce and not significantly different across farms or across treatments within a farm (Table 1).

All the farms participating in the microbial load study used potable water to wash vegetables. Five of these farms spin-dried leafy greens, and the others drip-dried them. Cooler temperatures varied from 0°C to 7.4°C.

Table 1.

Mean Total Coliform Counts of Leafy Greens Samples Collected from Different Locations at Various Minnesota Farms

	Farm (number of total samples analyzed)							
Sampling location	**1 (13)**	**2 (15)**	**3 (29)**	**4 (29)**	**5 (15)**	**6 (55)**	**7 (20)**	**8 (37)**
Field	1.4 ± 1.5	2.7 ± 1.5	2.0 ± 1.6	1.9 ± 1.1	0.8 ± 1.8	1.4 ± 1.5	0.9 ± 1.2	2.8 ± 0.2
Wash water without sanitizer	0.2 ± 0.4	3.4 ± 0.5	3.7 ± 0.3	1.4 ± 1.5	no sample	1.9 ± 1.5	no sample	3.5 ± 0.3
Wash water with sanitizer	no sample	2.4 ± 1.4	2.9 ± 0.6	no sample	2.0 ± 1.8	0.8 ± 1.2	0.6 ± 1.1	2.3 ± 2.1
Cooler	0	no sample	no sample	1.9 ± 1.1	0.6 ± 1.3	3.4 ± 0.4	no sample	1.9 ± 1.2

Farmers' market stall	no sample	no sample	no sample	0.1 ± 0.4	no sample	no sample	no sample	2.7 ± 0.5

Note. Units of mean total coliform counts are log colony forming units per gram of fresh lettuce tissue ± standard deviations.

In 2015, no *E. coli* was detected in any of the farm samples, despite farmers' use of animal manure as a soil amendment. However, all of the manure was either composted or applied to soil at least 120 days prior to harvest. A majority of the farms had free-roaming chickens, pets, or wild animals. *Salmonella* was detected in only one out of five samples from one farm's field. *Listeria* was detected in samples from two farms, but none were positive for *L. monocytogenes*. Coliform counts of 2015 samples were all below 2 log CFU/g, and did not differ across treatments in any of the farm samples (data not shown). The farmers used composted dairy cow, chicken, or goat manure or peat as soil amendments. All farmers washing greens used potable water without added sanitizer and spin-dried greens.

Salmonella, *E. coli*, and *Listeria* (none *L. monocytogenes)* were detected on a few samples obtained from farmers' markets (Table 2). Total coliform counts were slightly higher on some farmers' market samples compared to samples collected directly from farms but still were less than 4 log CFU/g. Only one farmers' market vendor fertilized leafy greens. All vendors washed leafy greens in potable water, but only one spin-dried greens.

Table 2.

Levels of Indicator Microbes on Minnesota Farmers' Market Leafy Greens Samples

Farmers' market vendor	Mean coliform count (log CFU/g) ± *SD*	*E. coli* (no. positive/total)	*Salmonella* (no. positive/total)	*Listeria* (no. positive/total)
A	3.1 ± 0.1	2/3 (mean 13 CFU/g)	0/3	3/3
B	3.2 ± 0.2	0/3	0/3	2/3
C	3.3 ± 0.1	0/3	0/3	0/3
D	2.4 ± 0.7	0/3	1/3	0/3
E	0.6 ± 1.1	0/5	0/5	3/5
F	1.0 ± 1.2	0/5	0/5	0/5
G	0.1 ± 0.2	0/5	0/5	0/5

Discussion and Conclusions

The lack of detection of *E. coli* on leafy greens collected from farms in our study contrasts with the results of a study conducted in 2002 (Mukherjee, Speh, Dyck, & Diez-Gonzalez, 2004). Mukherjee et al. (2004) detected *E. coli* on nine out of 65 samples of leafy greens and 12 out of 39 lettuce samples from conventional farms. Our study collected samples from two farms that also participated in the Mukherjee et al. (2004) study. One farm has organic certification and had normal ranges of coliforms and no detectable *E. coli* or *Salmonella* in both our study and the Mukherjee et al. (2004) study. The other farm uses organic methods but is not certified and had more than 4 log CFU/g of coliforms and no detectable *E. coli* or *Salmonella* contamination in 2002 (Mukherjee et al., 2004). This farm had normal levels of coliforms and no detectable *E. coli* or *Salmonella* in our study. The farmers could not recall what changes they implemented between 2002 and 2014 but stated that they now use green manures in lieu of animal manure.

The low levels of bacterial contamination on leafy greens found in our study are similar to those reported by other researchers for small-acreage farms. Johnston et al. (2005) found less than 3 log CFU/g enterococci and coliforms on leafy greens from farms and packing sheds in the southern United States. Xu, Pahl, Buchanan, and Micallef (2015) did not detect any *Salmonella* or *Listeria* on leafy greens from seven Maryland farms over 2 years. Thunberg, Tran, Bennett, Matthews, and Belay (2002) detected no *Salmonella* and 0 to 6.5 $\log_{10}$ CFU/g total coliforms on lettuce from farmers' markets and retail supermarkets in the Washington, DC, area. Wood, Chen, Friesen, Delaquis, & Allen (2015) collected samples from farmers' markets in Vancouver, British Columbia, and found mean coliform levels of 1.9 log CFU/g.

The microbial levels reported in our study suggest that locally grown leafy greens in Minnesota have low risk of causing illnesses. Although no significant differences were observed in coliform levels between samples washed in water with and without sanitizer, we recommend that farmers use sanitizer in wash water to ensure that bacterial levels are kept low. Blevins and Grubinger (2012) reported that SaniDate 5.0 added to wash water was effective at reducing *E. coli* levels and recommended that farmers add sanitizer to the first of three rinses of produce. On the other hand, although most of the produce tested by Johnston et al. (2005) was washed in chlorinated water, the small microbial loads on the produce were not further reduced by washing.

Although we reported total coliform levels, this parameter should not be used alone as indication of fecal contamination. Many types of coliforms occur naturally on plants and may be false-positive indicators of fecal contamination (Doyle & Erickson, 2006). Confirmatory tests for *E. coli* are recommended.

With these results in mind, we can refine the focuses of our future GAP education. New FSMA standards for produce emphasize water quality; biological soil amendments; animals; worker training, health, and hygiene; and equipment, tools, and buildings. Most of the farmers participating in our study used potable water, did not use raw manure, have few employees, and provided toilet and hand-washing facilities. Therefore, in future outreach programming, we can emphasize worker hygiene and farming operation cleanliness.

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