

Evaluation of Good Agricultural Practices (GAP) Compliance by Small Farmers in Kentucky: Assessing Microbial Quality of Produce

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Abstract

In recent years the number of bacterial food-borne outbreaks associated with contaminated produce has increased substantially. *Escherichia coli* (*E.coli*) continues to contribute to the majority of foodborne illnesses. With more small farmers starting organic production and given a wide range of organic production practices, there is a vulnerable segment which demands continuous microbial safety assessments. In the current study, twenty small produce farms from fourteen counties in Kentucky participated in a survey outlining farmers' procedures during their routine operations. These farms were visited three times, during the pre-growing, harvest, and post-harvesting seasons. A total of 59 produce samples were collected from 16 organic and 4 conventional farms, respectively. No differences were observed in the percentage of produce contaminated with *E. coli* between the organic (25%) and conventional (26.3%) practices. However, 45.5% of produce grown at and below the surface was contaminated with *E.coli* while 13.5% of the produce that grows above the surface was found to be contaminated. Most of the contamination was correlated with fields that were fertilized with manure in the past 90 days or less. Findings of the study were shared with participating farmers and they were counseled on Good Agricultural Practices.

Keywords: Small and Limited Resource Farmers, GAP compliance, Fresh produce, Microbial quality, E. Coli, Listeria

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1. Introduction

American consumers are becoming more aware of the importance of healthy, safe, and fresh produce (Prance, 2007). Several government and non-government agencies, including Public Health advocates have been campaigning to the public to consume more fresh produce. In 2010 the USDA created a new set of Dietary Guidelines for all Americans. Consistent with these guidelines is the new myPlate, replacing the historical Food Pyramid. The myPlate guideline recommends filling up half of the plate during each meal with fruits and vegetables instead of eating two to three servings of fruits and vegetables per day, as was recommended by the Food Pyramid (USDA Center for Nutrition Policy and Promotion, 2014).

As the market for fresh produce continues to rise, the number of foodborne outbreaks associated with contaminated produce has risen dramatically. Many types of produce are ready-to-eat and are meant to be consumed "as is," without any additional processing, such as cooking or heating. The lack of added processing prevents any opportunity for consumers to eliminate surviving pathogens on the produce which elevates the risk of foodborne outbreaks. Therefore, the microbiological safety of produce has become one of the most critical challenges for growers, producers, retail vendors, and consumers.

The top five most concerning foodborne pathogens associated with recent foodborne outbreaks involving fresh fruits and vegetables were *Listeria monocytogenes*, *Salmonella* (non-typhoidal serotypes only), *Escherichia coli* (*E. coli*) O157:H7, *E. coli* non-O157 STEC, and *Campylobacter*. On average, *Listeria monocytogenes* is estimated to cause 1600 illnesses each year in the U.S. Of these illnesses, 1400 result in hospitalizations, and 250 result in deaths (Scallan et al., 2011). Although foodborne outbreaks from *Listeria* are not as common as *Salmonella*, they cause national news because most listeriosis infections are caused by lack of proper food management and result in outbreaks that have serious consequences (Todd, 2013). In 2011 a large multi-state foodborne outbreak of listeriosis occurred in 28 states in the United States, and was associated with contaminated cantaloupes. This foodborne pathogen caused 147 reported illnesses, 33 deaths, and one miscarriage (Center for Disease Control and Prevention, 2012). Also in 2011 a multi-state foodborne outbreak of *Salmonella* occurred in 25 states and was associated with contaminated papayas. This outbreak reportedly caused a total of 106 individuals to become ill (Center for Disease Control and Prevention, 2011).

In 2012 another multi-state foodborne outbreak occurred, which was associated with contaminated cantaloupes, but this time two strains of *Salmonella*, *Salmonellatyphimurium* and *Salmonellanewport*, were implicated. These two strains caused a total of 261 people to become ill across 24 states. In all, 94 people were hospitalized, and 3 deaths occurred in Kentucky (Center for Disease Control and Prevention, 2012).

In addition to costing human lives, foodborne outbreaks have an economic cost to the industries and the nation, which typically run in billions of dollars. A study conducted by Produce Safety Project at Georgetown University in 2010 found that foodborne illness costs the United States a total of \$152 billion per year. Of this amount, it is estimated that foodborne illnesses associated with contaminated produce cost the U.S. roughly \$39 billion per year (Ferguson, 2010). The foodservice industry is not immune to these costs. On average, a single foodborne outbreak can cost a restaurant \$100,000 and a 30% reduction in yearly sales (Grover and Dausch, 2000). Even food production companies fortunate enough to detect contaminated products before an outbreak occurs are not immune to the costs associated with recalls. In July of 2014, Wawona Packing Company, located in California, issued a nationwide recall on several brands of plums, peaches, and nectarines from one of their manufacturing plants after discovering the fruit may have been contaminated with *Listeria monocytogenes* (Miller, 2014). In 2011 Grocery Manufacturers Association, Covington & Burling LLP, and Ernst & Young LLP., surveyed several corporate food production companies regarding the actual costs of a recall. They found that 77% of the participating companies that faced a food recall between 2005 and 2010 reported their estimated financial cost was up to \$30 million (Grocery Manufacturers Association, 2011). Therefore, it is critical to have a concerted collaboration from every aspect of the food chain to tackle the continued threat that foodborne outbreaks cause to the food, agriculture, economic, and public health systems.

The U.S. organic food market has increased steadily over the past five years, at a rate of 10 to 20 percent or more annually (Organic Trade Association, 2011). In 2012 industry analysts estimated that the U.S. organic food market had profited a total of \$28 billion in gross sales, which was an 11 percent increase from 2011. Forty-three percent of these profits came from the sales of organic produce (Greene, 2013). Organic food products can also sell anywhere from twenty to one-hundred percent more than their conventional counterparts (White, 2010).

Because of this growing popularity and the increased selling price for organic products, many farmers have adopted organic farming (Fan et al., 2009). As of 2013, there are 27,110 USDA certified organic farm operations in the world (USDA-AMS, 2014). Further, many consumers believe that organic produce is healthier and safer than conventionally-grown products. A study conducted in 2009 called *2009 U.S. Families' Organic Attitudes and Belief Study*, surveyed beliefs and attitudes regarding perceived health benefits of organic foods in United States households. Seventy-three percent of the U.S. families surveyed reported purchasing organic products at least occasionally, and stated they did so primarily for health reasons (Saulo, 2014). However, much of the information that consumers receive about food safety, food health, and farming is obtained from the media, and not from farmers or that is based on sound science. Most consumers don't know the real differences between conventional and organic farming (Organic Trade Association, 2010).

There are a number of small and mid-sized farms in Kentucky moving toward organic production techniques. Many of these farms previously grew tobacco, but have switched to harvesting organic or naturally-grown produce. Kentucky grew from having almost no certified organic farms in 2000 to 70 certified organic farms in 2011 and 42 in organic production but exempt from certification, and 49 in transition. (USDA National Agricultural Statistics Service, 2012). However, there are many more non-certified organic farms in Kentucky. All of these farms make \$50,000 or less annually from their products. These farms use some organic production methods to harvest their produce but, unlike the certified organic farms, they do not have to abide by the standards and requirements set by the National Organic Program (National Organic Program, 2014).

Farmers markets have also become very popular in the United States. From 1994 to 2004 the number of farmers markets in the United States increased from 1,755 to more than 3,700. During the same timeframe, farmers markets in Kentucky also tripled. As of 2013 the USDA Agricultural Marketing Service documented 8,144 farmers markets in the United States (USDA-AMS, 2013). The 2014 USDA Farm Bill increased funding for farmers markets by \$20 million annually for its Farmers Market and Local Food Promotion Program (USDA, 2014). Many non-certified organic, small, and limited-resource farmers sell at farmers markets. In 2008 81% of all local farmer's market food sales were from small farms that averaged less than \$50,000 in gross annual sales (Low and Vogel, 2011).

Although consumers reported that they shopped at farmers markets because of the shopping atmosphere, their environmental consciousness, the product freshness, and their desire to support local farmers (Gao et al., 2012), Kentucky customers overwhelmingly reported that they shopped at farmers markets largely for the quality of the produce (Ernst and Woods, 2010). Most certified and non-certified organic farmers are just as concerned about the food safety and nutritional quality of their products, as they are about environmental concerns regarding energy use, soil erosion, and water quality. However, data regarding the microbiological safety of organic produce are relatively limited, probably largely due to a wide range of organic production practices.

The greatest sources of fertility for organic production come from plant compost and animal manure. The USDA National Organic Program states that treated animal manure must be composted to reach an internal temperature range of 55° -77 °C. However, untreated animal manure may be used if it is applied at least 90-120 days before harvesting the product, depending on whether the product could come in contact with the soil (Agricultural Marketing Service, 2000). In 2004 a nationwide survey was conducted on organic producer management practices, and reported that 43% of the respondents indicated that they never used untreated manure, and 22% stated that they applied untreated manure regularly (Mukherjee et al., 2004). The prevalence, levels, and survival of pathogens in animal manures as well as in composts are determined by a number of factors such as the age of the animal (higher in young animals), dietary changes in the animal (raising the fiber content or fasting), or other forms of stress the animal experiences in its lifetime (Nicholson et al., 2005).

E. coli is a naturally growing bacteria found in the gastrointestinal tract of most warm-blooded animals. *E. Coli* can be isolated from the feces of most livestock species, and is especially prevalent in cattle manure. Though most strains of *E.coli* do not cause any disease, one of the strains of this bacteria, *E. coli* O157:H7, produces an endotoxin, called Shiga toxin, which can cause serious illnesses. In 2013 a multi-state foodborne outbreak of *E. coli* O157:H7 occurred in four states and was associated with packaged, ready-to-eat salads. Thirty-three people became ill with the infection, and two people developed Hemolytic Uremic Syndrome (HUS), a disease that can cause red blood cell destruction and kidney failure (Center for Disease Control and Prevention, 2013).

In September 2014 175 people from 25 states got sick from an *E. coli* O157:H7 infection from eating prepackaged Dole baby spinach. Six of these people were children from Kentucky who developed HUS syndrome and were hospitalized (Corrum, 2014).

As the nation has become more health-conscious and the demands for healthy, organically-grown produce have risen dramatically, Kentucky farmers have increased production, using as many resources they have available to do so. However, in states such as Kentucky and Tennessee, fruit and vegetable sales are small proportions of total farm revenue for most farms, and on average, farmers used 10,000 acres or less for growing produce (Hall et al., 2006). Many of these local farmers who sell at farmers markets do not make more than \$50,000 annually, and, as of now, are not required to follow the standards set by the FDA's Food Safety Management Act (U.S. Food and Drug Administration, 2014). Kentucky farmers may also not be aware of Good Agricultural Practices (GAP), a guideline for farm management to reduce the amount of pathogens on produce (Food and Drug Administration, 1998).

2. Objectives

The objective of the study was to determine the correlation, if any, between on-farm food safety practices (outlined in the survey), and the microbiological quality of the produce from the farm samples.

3. Materials and Methods

3.1. Farmer Recruitment and Survey

Consenting conventional, certified and non-certified organic small and limited-resource farmers were contacted and recruited through a mailing list from Kentucky State University's current outreach and extension programs, such as the Small Farm Program, Third Thursday Thing, the Socially Disadvantaged Farmer Outreach Project and the Organic Association of Kentucky. Each farmer was given a survey that explored details regarding certification status, fertilization practices (such as the type(s) of manure or compost and/or chemicals, age of the manure or compost and the time of application), the source of irrigation water, surrounding land use, handling practices during harvesting, post harvesting and handling practices such as washing, packaging and storage.

Further, the survey was targeted to help in ascertaining the needs and gaps in knowledge of the farmers in various aspects of 'on farm' food safety. The information collected from the survey was used to identify associations with the microbiological results.

3.2. Sample Collection

During May to September 2014, twenty farmers, of which 4 grew conventional produce, 2 grew certified and 14 grew non-certified organic produce, participated in the study and were visited 1 to 3 times, depending on availability. The list of vegetables and fruit collected included tomatoes, Swiss chard, Japanese greens, lettuce, cabbage, green peppers, jalapeno peppers, banana peppers, broccoli, cucumbers, summer squash, green beans, green onions, eggplant, carrots, potatoes, cantaloupe, watermelon, apples, grapes, strawberries, blackberries, peaches, pears, and plums. During each visit, using aseptic techniques, 1 to 2 samples of produce 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 and fruit was less than 100 grams. Each farm was assigned a coded ID number, and samples were labeled with the date, immediately placed in a cooler, and transported to the laboratory (Frankfort, KY) for analyses. Samples were kept stored on metal shelves in the refrigerator until analysis began.

3.3. Microbial Analysis

Microbial analyses were started within 24 hours of receiving the samples. For lettuce, Japanese greens, Swiss chard, and cabbage, representative leaves from the exterior and interior sections were cut up and used to make 10 grams. Using stringent aseptic conditions, ten grams of sample was then mixed with 100 milliliter (mL) of Lauryl sulfate tryptose broth (LST), taken in a stomacher bag for 5 min, at 230 RPM. The mixture was then placed in 9 mL of LST, in serial dilutions up to 10^{-4} . One mL of the serial dilution of 10^{-3} was plated onto *E. coli*, Coliform and *Listeria* Petrifilm plates (3M Company, Maplewood, Minn.) and 100 microliters of the serial dilution of 10^{-3} was plated onto Eosin Methylene Blue (EMB) agar plates in duplicate, and placed into the incubator at 37°C for 48 hours.

For the water, the coliform count was determined by the three-tube most-probable-number (MPN) system using three 10-fold serial dilutions in double strength Lactose broth and was incubated for 48 hours at 37°C. Lactose tubes showing growth and gas were plated onto Eosin Methylene Blue (EMB) plates in duplicate and incubated for an additional 48 hours.

Five grams of each soil sample was mixed and manually stirred with 50 mL of sterile saline. One milliliter of this mixture was pipetted into 9 mL of sterile saline serial dilutions up to 10^{-4} . One milliliter of the serial dilution of 10^{-3} was plated in duplicate onto three different types of Petrifilm plates, namely *E. coli*, Coliform and *Listeria*, and incubated at 37°C for 48 hours.

3.4. Statistical Analysis

On the Petrifilm plates, colonies with color and gas were recorded as positive for each respective type. These results, as well as the data from the survey, were recorded onto Microsoft Excel and analyzed using IBM SPSS statistical software using One-way ANOVA and Pearson Chi-Square statistics, when appropriate.

4. Results

A total of 16 certified and non-certified organic and 4 conventional farmers participated in the study by providing samples and answering questions about their management practices. Among the organic farms, 2 were certified by Kentucky Department of Agriculture and 14 were non-certified but reported using organic practices. Two out of the four conventional farmers reported using composted manure in addition to their chemical fertilizer and commercial pesticide, while the remaining conventional farmers only used commercial fertilizer. Of the 16 organic farmers, 7 reported using aged or composted animal manure regularly as the main source of fertilizer. The remaining 9 organic farmers did not apply fertilizer of any sort to their fields.

When testing for *E. coli*, the soil amendments were categorized in three groups based on the type of the fertilizer used: farms that used non-commercial fertilizers (including compost, animal manures, and compost mixtures), farms that used no additional fertilizer on their fields, and farms that only used commercial fertilizers on their fields (Table 1).

Of the farms that used non-commercial fertilizers, 43.4% had *E. coli* contamination on their produce samples. Of the farms that did not use fertilizer at all, 41.2% had *E. Colion* produce samples. When comparing the group that did not use any type of fertilizer to the group that used non-commercial fertilizers, there was no statistical difference of *E. coli* prevalence. However, none of the farms that used commercial fertilizer had *E. coli* contamination either on their produce samples, and this was statistically significant when compared with the other two groups.

Table 1.E: Colicount in Produce Samples Compared with the Types of Fertilizer Used on Fields Prior to Planting.*

		<i>E. coli</i> positive samples ^a	Total
Fertilization Group	Commercial	0 0.0% ^A	14 100.0%
	Non Commercial	46 43.4% ^B	106 100.0%
	None	14 41.2% ^B	34 100.0%
Total		60 39.0%	154 100.0%
		Value	df
Pearson Chi-Square		9.883	2
			Significance
			.007

^aPairs of data having different letters (A and B) were significantly different (P < 0.05).

*Pearson Chi-Square test was performed.

Water samples were collected from the participating farms. The majority of farms used only one water source for irrigation, but some farms had as many as two sources and as few as no sources to collect (i.e. they just used rain water to irrigate their crops). In total, three types of water sources were collected in the study: city water, surface water, and well water (Table2). City water was the least contaminated irrigation source with a mean of 1.0 cfu/100 mL, and surface water had the highest average amount of *Coliform* count at 673.3 cfu/100 mL.

Table 2. Coliform Count in Different Irrigation Water Used by the Farmers in the Study

Water Type	N	Coliform count (mean cfu/100 mL \pm SD) ^a	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
City	22	1.0 \pm 3.1 ^A	-.4170	2.3261
Surface	6	673.3 \pm 330.5 ^B	326.5005	1020.1661
Well	5	263.4 \pm 470.7 ^C	-321.0292	847.8292
Total	33	163.0 \pm 336.5	43.6552	282.2842

^aData of water types having different letters (A through C) were significantly different ($P < 0.05$) within the Coliform count.

*One-Way ANOVA test was performed for evaluation of statistical significance

Of the 7 organic farmers using manure or compost, 6 reported using material less than 90 days old. Of the 2 conventional farmers using manure or compost, only one reported using material older than 90 days. The distribution of the type of manure used by 10 farmers either alone or in combination was as follows: compost 8, chicken manure 4, goat manure 2, and horse manure 1. Five of these farmers used compost in combination with another manure type.

In all, the organic and conventional farmers provided 40 and 19 produce samples, respectively. Organic farms supplied an average number of 2.56 produce samples, and conventional farms supplied an average of 4.75 produce samples. The ten produce types that made up over 65% of the organic produce were squash, tomatoes, green beans, broccoli, carrots, lettuce, soybeans, strawberries, banana peppers and corn, and the remainder included apples, cabbage, cucumber, eggplant, green onion, potato, sorghum, Swiss chard, pear, cauliflower, shallot, cayenne, and cantaloupe. The most frequently collected conventional produce were apples, grapes, Japanese greens, peaches and squash.

Coliform bacteria were detected in 25.42% of all samples, and the overall average count in both organic and conventional produce was 1.6×10^4 cfu/g (Table 3). No statistical significances were found when the coliform counts of the produce varieties were compared between the two groups of farms.

Table 3: Coliform Counts in Produce Varieties from Conventional and Organic Growers

Produce variety	Coliform Count (mean cfu/g)	
	Organic	Conventional
Apple	1	0
Broccoli	0.8	n/a
Cabbage	0	n/a
Carrot	0	n/a
Cucumber	2	n/a
Eggplant	0	n/a
Grape	n/a	0
Green bean	0	20
Green onion	0	n/a
Jalapeno	n/a	0
Japanese green	n/a	11
Lettuce	0	n/a
Okra	n/a	0
Peach	n/a	0
Plum	n/a	0
Potato	0	n/a
Sorghum	0	n/a
Soybean	0	n/a
Squash	3.1	5.3
Swiss Chard	0	n/a
Tomato	0.4	0.5
Pear	0	0
Strawberry	0	n/a
Cauliflower	20	n/a
Blackberry	n/a	0
Shallot	0	n/a
Banana Pepper	0	n/a
Cayenne	0	n/a
Corn	0	n/a
Cantaloupe	0	n/a

n/a=sample not available

E. coli was isolated from 25.42% of all fruits and vegetables analyzed, and the average count of those samples that tested positive was 3.5 cfu/g.

The overall prevalence of *E. coli* was about the same in conventional and organic fruits and vegetables (Table 4). However, statistically significant changes were observed in the distribution of *E. coli*, depending on whether the produce was grown over or below the soil surface. Produce that grew below ground or contacting the soil surface had a higher chance of being contaminated with *E. coli* than produce that grew above ground and not in contact with the soil, and this was statistically significant (Table 5).

Table 4: Comparison of *Escherichia Coli* Prevalence in Produce Samples From Organic and Conventional Farms

Escherichia coli prevalence (%)			
Produce variety	Organic	Conventional	Total
Apple	0	0	0
Broccoli	100	n/a	100
Cabbage	0	n/a	0
Carrot	50	n/a	50
Cucumber	100	n/a	100
Eggplant	0	n/a	0
Grape	n/a	0	0
Green bean	0	100	25
Green onion	0	n/a	0
Jalapeno	n/a	0	0
Japanese green	n/a	100	100
Lettuce	0	n/a	0
Okra	n/a	0	0
Peach	n/a	0	0
Plum	n/a	0	0
Potato	100	n/a	100
Sorghum	0	n/a	0
Soybean	0	n/a	0
Squash	60	50	57.1
Swiss Chard	0	n/a	0
Tomato	20	100	33.3
Pear	0	0	0
Strawberry	0	n/a	0
Cauliflower	100	n/a	100
Blackberry	n/a	0	0
Shallot	0	n/a	0
Banana Pepper	0	n/a	0
Cayenne	0	n/a	0
Corn	0	n/a	0
Cantaloupe	0	n/a	0
Overall	25	26.3	25.4

n/a=sample not available

Table 5: Detection of Escherichia Coli in Produce Based on the Growing Location of the Produce

		<i>E. coli</i> positive samples ^a	Total samples
Location of Produce	Above soil surface	5 13.5% ^A	37 100.0%
	At or below soil surface	10 45.5% ^B	22 100.0%
Total		15 25.4%	59 100.0%
		Value	Df
Pearson Chi-Square		7.424	1
			Significance
			.006

^aPairs of data having different letters (A and B) were significantly different ($P < 0.05$).

*Pearson Chi-Square test was performed for evaluation of significance.

Five out of the six farms that indicated having used manure in 90 days or less prior to planting had *E. coli* contamination on their produce samples. Of the remaining 7 farms that used manure or compost mixture 90 days or more prior to planting, only 2 farms had produce testing positive for *E. coli*, and one of these farms was a conventional farm. *E. coli* was detected almost always on all produce from two organic farms and one conventional farm. Of these three farmers, only one reported having a food safety plan in the survey. However, all three of these farmers mentioned this year was the first year they had used animal manure to fertilize their fields. Of the remaining farms, 13 farms had no *E. coli* detected on their produce samples during the entire length of the study. None of the twenty farms had *Listeria* present on their produce.

5. Discussion

The intent of this study was to provide an assessment of the microbiological quality of fruits and vegetables typically grown at farms consisting of small scale production. Organically grown produce often relies on animal wastes as fertilizers, making organic produce seem as though they carry a greater risk for foodborne disease than conventionally grown produce (Brandt, 2012).

Few microbiological studies have assessed the microbial load on organic produce (Doyle, 2001, Mukherjee et al., 2004), but no study to date has conducted microbial analysis on produce from Kentucky at the farm level. By sampling during the pre-harvest season, the bacteria were likely influenced only by farm practices and individual farm environment.

The participating farms in this study used a wide variety of soil amendments, ranging from commercial fertilizers, to plant compost, to a variety of animal manures, including those from horse, chicken, and goat, to a mix between compost and animal manure. In addition, 9 farms did not apply additional fertilizer to their fields. In comparing these soil amendments to the results from the soil and produce samples that were collected, fertilizers were separated into three distinct groups: non-commercial fertilizers, which included farms that used plant compost, animal manure, or a combination of the two, no fertilizer use, and commercial fertilizer use. Of these three groups, 9 farms used non-commercial fertilizers, 9 farms did not use fertilizers, and 2 farms used commercial fertilizers prior to planting their produce.

E. coli has been used for years as the reference indicator for fecal contamination when conducting microbiological analysis for water and food products. Currently there is not a law regarding the amount of *E. Coli* allowed in water intended for agricultural use. However, in September 2014, the FDA announced proposed revisions to the Food Safety Modernization Act (FSMA) stating a limit on *E. coli* allowed in water intended for agricultural use at 235 cfu/100 mL (U.S. Food and Drug Administration, 2014).

Most of the water collected from the participating farms was from city water, which had an average of 1.0 cfu/100 mL of *E. coli*. The EPA has set a standard drinking water limit of *E. coli* of 0 cfu/100 mL, which applies to all municipal water in the country (EPA, 2013). Each city is required to test and treat municipal water monthly to ensure the city follows with this EPA regulation. Two farms had water with an *E. coli* level higher than the standard of 0. However, since the law requires the water to be tested monthly, this *E. coli* contamination level is likely due to an outside source (i.e. contaminated hose or faucet), rather than the water itself.

The well water, which was collected from 3 different farms, had an average *E. coli* contamination of 263.4 cfu/100 mL, and does not fall under the proposed FSMA standards (U.S. Food and Drug Administration, 2014).

However, two out of three farms had a contamination level exceeding the standards, at 460 and 1100 cfu/100 mL of *E. coli*, respectively.

The surface water had the highest average amount of *E. coli* contamination, and had an average contamination level of 673.3 cfu/100 mL. This consisted of three farms that collected rain water for drip irrigation, and one farm that used creek water to irrigate his crops. Of the farms that used rain water, one farm collected rain from rain barrels, one from two-gallon buckets, and one from the gutters off of high tunnels. None of these farmers mentioned cleaning the rain containers regularly.

The farm that used creek water for irrigation had a total *E. coli* contamination level of 1100 cfu/100 mL. This farmer had two branches of creek water running around his fields. One of these branches started away from his property on top of a hill that contained a large amount of cattle. As the cattle roamed through the creek, it is likely that their feces was carried along, through runoff, downstream toward the study farm, contaminating both branches of the creek. This farmer also had two other water sources for irrigating his crops, city water and natural rain from the sky. However, he also mentioned having a problem with his fields flooding when it rained, which allowed for the creek water to contaminate his fields.

In a similar study conducted in Minnesota, Mukherjee et al found the prevalence of *E. coli* in organic produce to be six times greater than in conventional produce (Mukherjee et al., 2004). However, in the current study no statistical significance in the prevalence of *E. Coli* between organic or conventional produce were observed. Only four farms surveyed were considered conventional, two farms were USDA certified organic farms and the remaining fourteen were non-certified organic farms. Of the four conventional farms, two indicated they preferred to use organic methods, and sprayed pesticide or used fertilizer only as a last resort. These two conventional farms also used animal manure as a fertilizer to amend the soil.

The FDA concludes that the top three produce most likely to be contaminated are sprouts (including green beans), leafy greens (including lettuce and spinach), and tomatoes (Food and Drug Administration, 2013). However, in this study, broccoli, cucumber, Japanese greens, potatoes, and cauliflower had the highest percentage of *E. coli* contamination. Squash and carrots had the second highest percentage of *E. coli* contamination.

Overall, 25.4% of the produce sampled from the 20 participating farms was contaminated with *E. coli*. Five out of the six farms that indicated they used manure in 90 days or less prior to planting had *E. coli* contamination on their produce samples, as well as those which recovered run-off water from upstream carrying animal feces, which indicates the link between Good Agricultural Practices (GAP) and the microbiological quality of fresh produce (Food and Drug Administration, 1998).

After the second visit, farmers were given recommendations consistent with the GAP standards tailored toward their specific needs to improve the quality of their produce in the future. Farmers were enthusiastic about these suggestions, asked further questions, and started brainstorming cleaner options for irrigation and fertilization on their fields.

It is not uncommon to find *E. coli* in soil, especially in soil amended with animal manure, which can remain in the ground for many years (Unc et al., 2006, Davis and Kendall, 2014). Laboratory studies suggest that *E. coli* can contaminate produce by colonizing near the roots and surfaces of the plant from already infested soil (Klerks et al., 2007). Therefore, it is likely that produce growing near the soil would contain a higher microbial load, including *E. coli*. In this study, 45.5% of the produce that grew at or below the surface of the soil contained *E. coli*, while only 13.5% of the produce that grew above the surface was contaminated. This indicates that there is a strong connection between the type of produce (root crops vs above ground plant parts) and the microbiological quality. Some farmers, aware of this fact, purposefully planted produce that grew above the surface of the soil so that the produce would have less of an opportunity to make contact with the ground in the areas where they had placed manure six months prior.

The results of this study do not support claims that organic produce contains a higher amount of *E. coli* than conventional produce. However, the observation that five out of six farms that used animal manure for fertilizer 90 days or less prior to planting had *E. coli* contamination on their produce supports the need for GAP training for all farmers (Food and Drug Administration, 1998). The results also suggest that minimizing *E. coli* contamination in other parts of the growing process, such as in the irrigation water, could greatly improve the microbial quality of the produce. Further analysis of the produce from the participating farms during the harvest and post-growing seasons is being studied and recorded to determine any changes from these results.

In addition, isolation of *E. coli* O157:H7, using CHROMagar, will be performed on all *E. coli* positive samples. We also are currently studying the antibiotic profiles of all contaminated samples for antibiotic resistance.

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7. References

- Ait Melloul, A., L. Hassani and L. Rafouk. 2001. Salmonella contamination of vegetables irrigated with untreated wastewater. *World Journal of Microbiology & Biotechnology*17:207-209.
- Agricultural Marketing Service. 2000. National Organic Program. Final Rule. 7 CFR part 205. U.S. Department of Agriculture, Washinton, D.C. Available at: <http://www.ams.usda.gov/AMSV1.0/nop>. Accessed 27 March 2014.
- Brackett, R. E. and Splittstoesser, D. F. 2001. Fruits and Vegetables, p. 515. In F. P. Downes and K. Ito (eds.), *Compendium of Methods for the Microbiological Examination of Foods*. American Public Health Association, Washington, DC.
- Brandt, Michelle. 2009. Little evidence of health benefits from organic foods, study finds. Available at: <http://med.stanford.edu/news/all-news/2012/09/little-evidence-of-health-benefits-from-organic-foods-study-finds.html>. Accessed 2 October 2014
- Burnett SL, Chen J, Beuchat LR. 2000. Attachment of *Escherichia coli* O157:H7 to the surfaces and internal structures of apples as detected by confocal scanning laser microscopy. *Applied Environmental Microbiology*66: 4679–4687.
- Bussard, Jesse. 2011. Kentucky Ag Facts: Cattle. Available at: <http://pearlsnapblog.com/2011/09/26/kentucky-ag-facts-cattle/>. Accessed 28 March 2014.
- Center for Disease Control and Prevention. 2011. Multistate Outbreak of Human Salmonella Agona Infections Linked to Whole, Fresh Imported Papayas. Available at: <http://www.cdc.gov/salmonella/agona-papayas/index.html>. Accessed 27 March 2014.
- Center for Disease Control and Prevention. 2012. Multistate Outbreak of Listeriosis Linked to Whole Cantaloupes from Jensen Farms, Colorado. Available at: <http://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/082712/>. Accessed 27 March 2014.

- Center for Disease Control and Prevention. 2012. Multistate Outbreak of Salmonella Typhimurium and Salmonella Newport Infections Linked to Cantaloupe (Final Update). Available at: <http://www.cdc.gov/salmonella/typhimurium-cantaloupe-08-12/index.html>. Accessed 27 March 2014.
- Center for Disease Control and Prevention. 2013. Multistate Outbreak of Shiga toxin-producing Escherichia coli O157:H7 Infections Linked to Ready-to-Eat Salads (Final Update). Available at: <http://www.cdc.gov/ecoli/2013/O157H7-11-13/index.html>. Accessed 28 March 2014.
- Corrum, Kerry. 2014. Officials Zero-in on E. coli Source. Available at: <http://www.14news.com/story/5432506/officials-zero-in-on-e-coli-source>. Accessed 16 September 2014.
- Davis, J.G. and P. Kendall. 2014. Colorado State University. Preventing E. coli From Garden to Plate. Available at: <http://www.ext.colostate.edu/pubs/foodnut/09369.html>. Accessed 2 October 2014.
- Doyle, M.P. 2001. Keeping foodborne pathogens down the farm. USDA Food Safety and Inspection Service. Available at: <http://www.fsis.usda.gov/OPPDE/animalprod/Presentations/KFPPF%20Aug%2001/>. Accessed 20 March 2014.
- EPA. 2013. Drinking Water Contaminants. Available at: <http://water.epa.gov/drink/contaminants/>. Accessed 2 October 2014.
- Ernst, Matt and Tim Woods. University of Kentucky Department of Agricultural Economics. 2010. Selling Farm Products at Farmers Markets. Available at: www.uky.edu/Ag/CCD/marketing/farmmarket.pdf. Accessed 26 March 2014.
- Fan, X., Niemira, B.A., Doona, C.J., Feehery, F.E., and R.B. Gravani. 2009. Microbial Safety of Fresh Produce. Blackwell Publishing and the Institute of Food Technologists. P. 83.
- Ferguson, Alex. Food Safety News. 2010. Foodborne Illness By The Numbers. Available at: http://www.foodsafetynews.com/2010/03/foodborne-illness-by-the-numbers/#.UzQxQmbD_IU. Accessed 27 March 2014.
- Food and Drug Administration. 1998. Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables. Available at: <http://www.fda.gov/downloads/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/UCM169112.pdf>. Accessed 2 October 2014.
- Food and Drug Administration. 2013. Standards for the Growing, Harvesting, Packaging, and Holding of Produce for Human Consumption; Proposed Rule. Available at: <http://www.fda.gov/downloads/Food/GuidanceRegulation/FSMA/UCM360734.pdf>. Accessed 1 October 2014.
- Freedom Kentucky. 2010. Kentucky Agriculture. Available at: http://www.freedomkentucky.org/index.php?title=Kentucky_Agriculture. Accessed 1 April 2014.
- Gao, Z.F., M. Swisher, and X. Zhao. 2012. A new look at farmers' markets: consumer knowledge and loyalty. Horticulture Science47:1102-1107.

- Greene, Catherine. USDA. Economic Research Service. 2013. Growth Patterns in the U.S. Organic Food Industry. Available at: http://www.ers.usda.gov/amber-waves/2013-october/growth-patterns-in-the-us-organic-industry.aspx#.UzMhHGbD_IU. Accessed 26 March 2014.
- Grocery Manufacturers Association. 2011. Capturing Recall Costs: Measuring and Recovering the Losses. Available at: http://www.gmaonline.org/file-manager/images/gmapublications/Capturing_Recall_Costs_GMA_Whitepaper_FIN AL.pdf. Accessed 2 September 2014.
- Grover, S.F. and Dausch, J.G. 2000. Hepatitis A in the foodservice industry. *Food Management*81:80-86.
- Guo, X. et al. 2001. Survival of *Salmonellae* on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. *Applied Environmental Microbiology*67:4760-4764.
- Hozzumi-Cho, Hozzumi and Motosu-gun, Gifu. 2001. Biphasic membrane effects of capsaicin, an active component in *Capsicum* species. *Journal of Ethnopharmacology*76(3):313
- Janisiewicz, W.J. et al. 1999. Fate of *Escherichia coli* O157:H7 on fresh-cut apple tissue and its potential for transmission by fruit flies. *Applied Environmental Microbiology*66:1-5.
- Kentucky Department of Agriculture. 2014. Grants and Funding Opportunities. Available at: <http://www.kyagr.com/marketing/grants.html>. Accessed 1 April 2014.
- Kentucky Horse Council. 2013. Kentucky's Horse Industry by the Numbers. Available at: www.kyhbpa.org/resources/HorseIndustryFactsandFigures.pdf. Accessed 28 March 2014.
- Kentucky Poultry Foundation. 2013. Kentucky Poultry Industry Facts. Available at: <http://www.kypoultry.org/pfacts/>. Accessed 28 March 2014.
- Kentucky State University Center for Sustainability of Farms and Families. 2014. Small Scale Farm Grant Program. Available at: <http://www.kysu.edu/landGrant/CSFF/ssfarmgrantprogram.htm>. Accessed 1 April 2014.
- Kentucky State University Land Grant. 2014. Small Farms. Available at: <http://www.kysu.edu/landGrant/coopextensionprogram/agricultureNaturalResources/smallfarms.htm>. Accessed 1 April 2014.
- Klerks, M. M., M. van Gent-Pelzer, E. Franz, C. Zijlstra, and A. H. van Bruggen. 2007. Physiological and molecular responses of *Lactuca sativa* to COLONIZATION by *Salmonella enterica* serovar. *Applied Environmental Microbiology*73:4905-14.
- Low, S.A. and S. Vogel. 2011. Direct and intermediated marketing of local foods in the United States. U.S. Department of Agriculture, Economic research report no. 128. Available at: http://www.ers.usda.gov/media/138324/err128_2_.pdf. Accessed 27 June 2013.
- Lynch, M.F., R.V. Tauxe, and C.V. Hedberg. 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology. Infection*.137:307-315.

- Miller, Brewster. 2014. Fruit Recall expanded by another 5 days to ensure safety for the public. Available at: <http://www.dumb-out.net/fruit-recall-expanded-another-5-days-ensure-safety-public/2703>. Accessed 5 August 2014.
- Mukherjee, A., Speh, D., Dyck, E., and Diez-Gonzalez, F. 2004. Preharvest Evaluation of Coliforms, *Escherichia coli*, *Salmonella* and *Escherichia coli* O157:H7 in Organic and Conventional Produce Grown by Minnesota Farmers. *Journal of Food Protection*67(5): 894-900.
- National Organic Program. 2014. Exemptions and Exclusions from Certification. Available at: <http://www.ecfr.gov/cgi-bin/text-idx?c=ecfr;sid=c1b7938d6e90785f50909e1dfeb5f772;rgn=div5;view=text;node=7%3A3.1.1.9.32;idno=7;cc=ecfr#7:3.1.1.9.32.2.354.2>. Accessed 28 March 2014.
- Nicholson F. A., Groves S. J., Chambers B. J. 2005. Pathogen survival during livestock manure storage and following land application. *Bioresour. Technol.*96:135–143.
- Nitrogenmuvek Company, Ltd. 2008. Nitrogen Fertilizers. Available at: http://www.nitrogen.hu/nat/index.php?option=com_content&view=category&layout=blog&id=4&Itemid=235&lang=en. Accessed 2 October 2014.
- Organic Trade Association. 2010. Consumer Profile Facts. Available at: <http://www.ota.com/organic/mt/consumer.html>. Accessed 27 March 2014.
- Organic Trade Association. 2011. Industry Statistics and Projected Growth. Available at: <http://www.ota.com/organic/mt/business.html>. Accessed 26 March 2014.
- Penteado AL, Eblen BS, Miller AJ. 2004. Evidence of *Salmonella* internalization into fresh mangos during simulated postharvest insect disinfestation procedures. *Journal of Food Protection*67: 181–184.
- Plath, Pernell. 2013. Looking for the post-tobacco king of Kentucky agriculture. Available at: http://www.newfarm.org/features/0504/ky_tobacco.shtml. Accessed 1 April 2014.
- Prance, L. 2007. Increased health awareness drives organic food and beverage market. Available at: <http://www.foodnavigator.com/Market-Trends/Increased-health-awareness-drives-organic-food-and-beverage-market>. Accessed 8 October 2014.
- Saulo, Aurora A. 2014. It's Not All about the Science. *Food Safety Magazine*. 66.
- Scallan, E., R.M. Hoekstra, F.J. Angulo, R.V. Tauxe, M.A. Widdowson, S.L. Roy, J.L. Jones and P.M. Griffin. 2011. Foodborne illness acquired in the United States – Major Pathogens. *Emergent Infectious Disease*17:7-15.
- Schuenzel, K.M. and M.A. Harrison. 2002. Microbial antagonists of foodborne pathogens on fresh, minimally processed vegetables. *J. Food Prot.* 65:1909-1915.
- The Fertilizer Institute. 2014. U.S. Fertilizer Production. Available at: <http://www.tfi.org/industry-resources/fertilizer-economics/us-fertilizer-production>. Accessed 2 October 2014.
- Todd, Ewen C.D. 2013. The Burden of Listeriosis. *Food Safety Magazine* 28-35.
- Torriani, S., C. Orsi, and M. Vescovo. 1997. Potential of *Lactobacillus casei*, culture permeate, and lactic acid to control microorganisms in ready-to-use vegetables. *J. Food Prot.* 60:1564-1567.
- Tyler HL and Triplett EW. 2008. Plants as a habitat for beneficial and/or human pathogenic bacteria. *Annual Review of Phytopathology*46: 53–73
- Unc, A., J. Gardner, and S. Springthorpe. 2006. Recovery of *Escherichia coli* from Soil after Addition of Sterile Organic Waste. *Applied Environmental Microbiology*72(3):2287-2289.

- USDA. 2014. USDA 2014 Farm Bill Highlights. Available at: www.usda.gov/documents/usda-2014-farm-bill-highlights.pdf. Accessed 8 April 2014.
- USDA-AMS. 2013. Farmers Market Facts and Statistics/ Farmers Market Growth: 1994-2009. Available at: <http://www.ams.usda.gov/AMSV1.0/FarmersMarkets>. Accessed 26 March 2014.
- USDA-AMS. 2014. 2013 List of certified USDA organic operations. Available at: <http://apps.ams.usda.gov/nop/>. Accessed 27 March 2014.
- USDA. Center for Nutrition Policy and Promotion. 2014. MyPlate and Historical Food Pyramid Resources. Available at: <http://fnic.nal.usda.gov/dietary-guidance/myplate-and-historical-food-pyramid-resources>. Accessed 26 March 2014.
- USDA National Agricultural Statistics Service. 2012 Organic Agriculture. Available at: http://www.agcensus.usda.gov/Publications/2012/Full_Report/Volume_1,_Chapter_2_US_State_Level/st99_2_042_042.pdf. Accessed 27 March 2014.
- U.S. Food and Drug Administration. 2014. Proposed rule; supplemental notice of proposed rulemaking. Available at: <http://s3.amazonaws.com/public-inspection.federalregister.gov/2014-22447.pdf>. Accessed 30 September 2014.
- Weil, Andrew. 2010. Ammonia in Ground Beef? Available at: <http://www.drweil.com/drw/u/QAA400685/Ammonia-in-Ground-Beef.html>. Accessed 2 October 2014.
- White, Annie. 2010. Why Organic Food Costs So Much. Available at: <https://www.mint.com/blog/trends/organic-food-07082010/>. Accessed 27 March 2014.
- Zhuang RY, Beuchat LR, Angulo FJ. 1995. Fate of Salmonella montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. *Applied Environmental Microbiology* 61: 2127–2131.