



**CPS 2013 RFP  
FINAL PROJECT REPORT**

**Project Title**

Food safety risks at the fresh produce-animal interface: Identifying pathogen sources and their movement on diversified farms

**Project Period**

January 1, 2014 – December 31, 2015 (extended to January 31, 2016)

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**Objectives**

1) *Conduct a controlled study to determine the impact of buffer zone distances, temporal factors, air and insect on the movement of indicator (fecal coliforms, E. coli and Enterococcus sp.) and pathogenic (Salmonella, STEC O157:H7 and non-O157:H7 STEC) organisms at the animal:produce interface on the Piedmont Agriculture Research Station reflective of diversified farming systems.*

Siddhartha Thakur, NCSU

*Food safety risks at the fresh produce-animal interface: Identifying pathogen sources and their movement on diversified farms*

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*2) Validate the outcome of the first objective by studying the movement of pathogenic microorganisms from known animal reservoirs and potential environmental sources into fresh produce field in commercial diversified farms.*

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## FINAL REPORT

### Abstract

Sustainable farming practices have, at the core of their management systems, an integration of crops and livestock, recycling of nutrients, management of soil health and the well-being of the environment, crops, animals, farmers and consumers. While this practice increases productivity, there are potential risks that relate to microbial food safety. The interface of food animals and fresh produce in agricultural production is an area in need of information that could potentially reduce the risk of pathogen transmission and fresh produce contamination. The primary goal of this project was to determine the potential transmission of *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 and non-O157:H7 STEC from animal operations that are in close proximity to vegetable production systems on an experimental research station (Objective 1) and on commercial diversified farming operations (Objective 2).

At the Piedmont Agriculture Research Station, we studied the transmission and impact of buffer zone distances, temporal factors, and air on the movement of indicator (fecal coliforms, *E. coli* and *Enterococcus* spp.) and pathogenic (*Salmonella*, STEC O157:H7 and non-O157:H7 STEC) organisms at the animal (dairy and poultry):produce interface. We consistently isolated both *E. coli* and STEC from produce, soil, and animal fecal matter, but none from air samples. *Salmonella* was only isolated from poultry fecal samples on two occasions. However, no *Salmonella* was detected on any produce sample during the study. Based on the outcome of the two-year sampling period at the research station where we collected animal feces, soil, air, and fresh produce at varying distances from the animal source (30–400 ft), our results suggest that the 400-foot buffer zone distance between the animal source and the produce field proposed by the California LGMA rules may not be appropriate and that this buffer zone distance requirement needs to be revised and other barriers may need to be included to reduce pathogen transfer.

For our second objective, we collected farm samples, including produce, animal, and environmental, from commercial sustainable farms in North Carolina (NC, n=2) and Tennessee (TN, n=5) over a two-year period. There were clear distinctions between the outcomes based on the geographic location of these farms in the two states. Overall, *Salmonella* was isolated only in TN and not in NC from multiple samples. None of the fresh produce samples tested positive for *Salmonella* in either of the two states. However, STEC in TN was isolated from fresh produce, animal feces, environmental sources, and flies, while in NC only the fecal and soil samples tested positive. Though the focus of our project was on pathogen transfer from animal operations to diversified vegetable farming systems, our findings and suggestions are not limited to these agricultural environments since other cropping systems, including standard organic production, conventional farming practices, and biodynamic approaches, will also pose the risk of pathogen transfer when in close proximity to animal operations. Under this scenario, the proximity of an animal operation to produce fields seems to impact pathogen transfer to produce, and these parameters are irrespective of farming approaches. Consequently, whether farmers are dealing with fruits, vegetables, tree nuts, horticulture, or other crops included within the definition of "specialty crops," our findings and potential approaches to reduce pathogen transfer could benefit all.

### Background

One way to reduce the burden of illness due to consumption of contaminated fresh produce is to identify the different sources of contamination. Even following major advances and improvements in the field of microbial food safety, foodborne diseases occurring due to consumption of contaminated fresh produce remain one of the leading public health concerns. Contamination of leafy greens and melon fruit with human pathogens can occur anywhere throughout the production chain. The interaction of typically host

enteric-adapted bacterial pathogens with fresh produce grown in fields is complex and can occur due to multiple pathways, including: 1) the application of inadequately composted or raw animal waste as manure (Natvig et al., 2002; Santamaria and Toranzos, 2003); 2) surface water contamination via runoff from nearby food animal farms (Berger et al., 2010); 3) use of overhead irrigation instead of drip irrigation (Mitra et al., 2009); and 4) pathogen transmission via insects and other animals (Talley et al., 2009). A comprehensive study examining the transfer of these pathogens from livestock to fresh produce farms is lacking; this is especially true in diversified farms where animal operations are, by design, in close proximity to fresh produce, and growers frequently move between the two production environments. Sustainable farming systems operate under the premise of maximizing resource utilization and, under this precept, the recognition that waste generated in one system can be reclaimed in another system. This includes waste generated from animal operations to provide adequate nutrients to crops, a practice that could potentially increase the risk of produce contamination and foodborne illnesses. The identification of these transmission pathways on farms that rear animals and grow fresh produce in close proximity will help us to develop effective control measures and prevent fresh produce-associated outbreaks.

## Research Methods and Results

### Methods

#### Isolation of Non-Pathogenic and Pathogenic Microorganisms

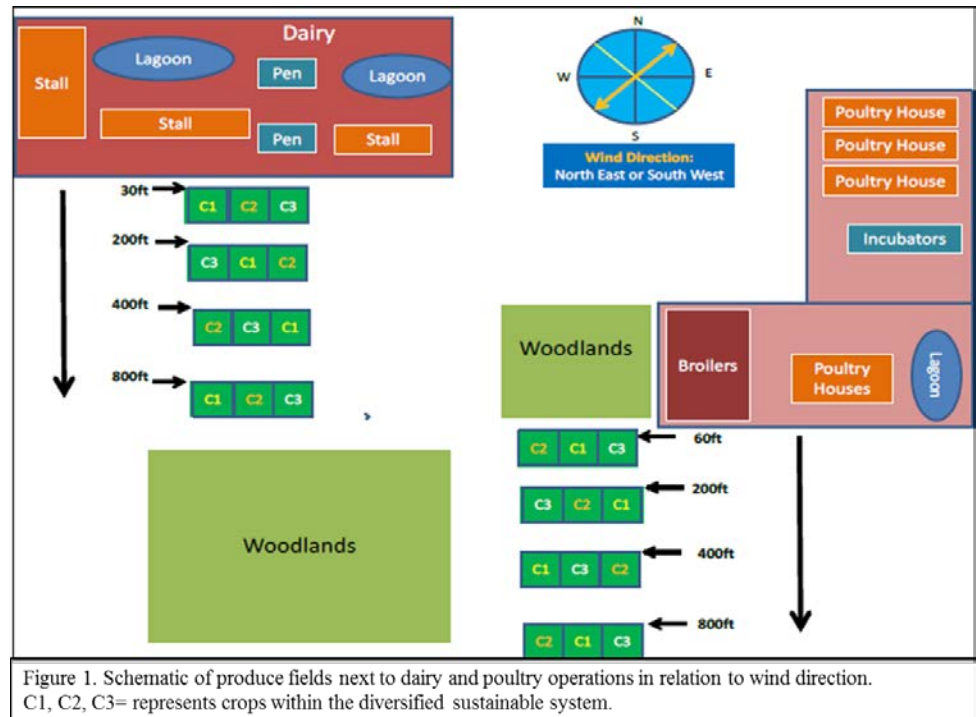
- a) Non-pathogenic *E. coli* and total coliform isolation: From the re-suspended pellet, serial ten-fold dilutions were prepared using 9 ml PPB tubes and plated on ChromECC; the plates were incubated for 24 h at 37°C before mauve and blue colonies were counted for coliforms and *E. coli*, respectively.
- b) *Salmonella* spp. isolation (produce only): From the re-suspended pellet, 50 ml were enriched with 100 ml of (2x) UPB for 18 h at 37°C. After enrichment 10 ml were transferred to 90 ml Tetrathionate Bile Broth (TBB) + iodine and incubated for 6 h at 42°C. After enrichment 10 ml were transferred into 90 ml mBroth bags and incubated for 24 h at 37°C. The mBroth enrichments were streaked on Xylose Lactose Tergitol 4 (XLT4) agar plates. Black colonies on this media were further isolated and purified on five occasions before molecular identification was performed using probe-based PCR as described by McEgan et al.(2013). The presence of *Salmonella* was only assessed on soil and produce samples grown in close proximity to layer-hen houses.
- c) STEC isolation: From the re-suspended pellet, 50 ml were enriched with 100 ml (2x) of UPB for 18 h at 37°C. After enrichment, 10 ml were transferred into 90 ml of (2x) mEHEC and incubated at 42°C for 24 h. After incubation the enrichment was streaked on ChromSTEC (25% supplements) and incubated at 37°C for 24 h. Mauve colonies present on ChromSTEC plates were further isolated and purified on five occasions before molecular identification was performed using probe-based PCR as described by Mitra et al. (2009) and Hoefler et al. (2011). The presence of STEC was not assessed on produce samples located in close proximity to poultry operations because the focus of these samples was *Salmonella* spp.
- d) *Enterococci* isolation: Quantification of *Enterococcus* spp. was achieved following the procedure by Haugland et al. (2005) using probe-based real-time PCR.
- e) Phenotypic and genotypic characterization: The Kauffman-White scheme was followed for serotyping; the isolates were shipped to the National Veterinary Services Laboratory (Ames, Iowa) for serotyping. Genotypic characterization and clonal relationships among *Salmonella* and STEC isolates were determined by PFGE using the PulseNet protocol. Analysis was performed using Bionumerics 5.0 (Applied Maths, Belgium) and the patterns compared via the Dice coefficient and UPGMA method (Ribot et al., 2006). Pathogen prevalence from different sources was compared using a Student's *t*-test, where applicable.  $P < 0.05$  was considered statistically significant.

### Objective 1 – Experimental Layout at the Piedmont Research Station

We set up the experimental layout at the Piedmont Research Station as shown below (**Figure 1**). This layout allowed us to compare the prevalence of indicator and pathogenic microorganisms between these two cropping systems and determine the overall environmental differences within them that could potentially increase contamination of fresh produce with pathogenic microorganisms. The field experimental layout consisted of 3 different vegetable plots each with seven to nine 50-ft rows containing seven to nine different crops per row. Crops included but were not limited to cabbage, turnips, green beans, romaine, red-oak lettuce, spinach, arugula, broccoli, cilantro, and bell peppers.

Each crop was harvested at least on two occasions; when microbial populations were similar between sampling points, data were combined to assist in data interpretation. Sampling also took into consideration water used for sprinkler or drip irrigation, both coming from well water. The plot selected next to the dairy operation was not in cultivation for the past 3 years while the plot located near the poultry unit was not used for any vegetable production for over 10 years. Both did not receive

any manure applications prior or during our experiment. Both locations were managed under organic practices except for fertilizer applications that were based on NPK chemistries and crop needs. Glyphosate applications were needed on two occasions to control pigweed



### Objective 2 – Commercial Farms

Commercial sustainable farms were recruited in NC (n=2) and TN (n=5). Specific examples of a typical farm layout are shown in the Appendix (**Figure A1**). Produce samples collected on the farms depended on the individual farm type, geographic location, and season. We also collected animal feces, soil, compost, and water samples from these farms. Collection of air samples was restricted to one farm in NC and this was dependent on the availability of air samplers and a power source. Only the pathogenic organisms *Salmonella*, non-O157:H7 STEC and STEC O157:H7 were isolated from these farms.

### Sample Analysis

Feces and manure analysis (*Salmonella* and STEC isolation) (n=200): Manure sampling from dairy operations took place in six different locations including the dry lot, the heifer barn, three locations within the dairy barn (lane 1, 2 and 3), and at the calf lot. At the poultry house, five different locations were sampled within a layer-hen house. Samples (150 g) of freshly excreted manure were collected from each operation with sterile plastic scoops and Whirl-Pak bags. All samples were refrigerated upon collection and transported back to the laboratories for processing.

Water analysis (n=72): Water sampling was performed monthly and consisted of collecting six 1-gallon samples at the outlet of the irrigation system within dairy and poultry operations. The experimental procedure followed for generic *E. coli*, Total coliforms and *Enterococci* were based on the procedure described by Sbodio et al. (2012). In brief, 1-gallon samples were filtered through a Modified Moore Swab system and the population of these microorganisms was determined by plating on ChromECC and M-*Enterococcus* media. The presence of *Enterococcus* was confirmed via PCR (Haugland et al., 2005).

Soil analysis (n=804): A similar procedure described by Gutierrez-Rodriguez et al. (2011) was followed to extract the potential *Salmonella* and STEC microorganisms from soil. In brief, 100–150 g of soil was placed into a 1-L Whirl-Pak bag and 200–300 ml of 0.02 M sodium phosphate + Tween 20 was added to assist in detaching cells from the silt-clay fraction. After mixing, the entire suspension was transferred into a new medium-size stand-up bag without filter and 250 ml of Universal Pre-enrichment Broth (UPB) at 2x concentration was added. Each sample was enriched for 18 h at 35°C, and the procedures listed below for *Salmonella* or STEC were followed to determine their presence in all the soil samples.

Produce analysis (n=1620): For each location (32, 200, and 400 ft) within field plots located in close proximity to dairy or poultry operations, a total of 7–9 replicates each of 150 g of produce were collected per distance and per crop. Crop rotation varied with growing season and included sampling of beans, cucumbers, red and green lettuce, turnips, squash, peppers, cabbage, and broccoli. All samples were first rinsed with 0.01M potassium phosphate buffer (PPB) supplemented with 0.05% Tween 20 (PPBT) at a 2:1 ratio (buffer/crop). Each bag was then vortexed for 30 s and the entire supernatant was pelleted and used for the determination of indicator microorganisms (total coliforms (TC), non-pathogenic *E. coli* and *Enterococcus* spp.), STEC (dairy locations) and *Salmonella* spp. (poultry locations). A portion of supernatant was used for DNA extraction and to describe microbial community composition and structure, and later used to compare microbiomes between dairy and poultry samples.

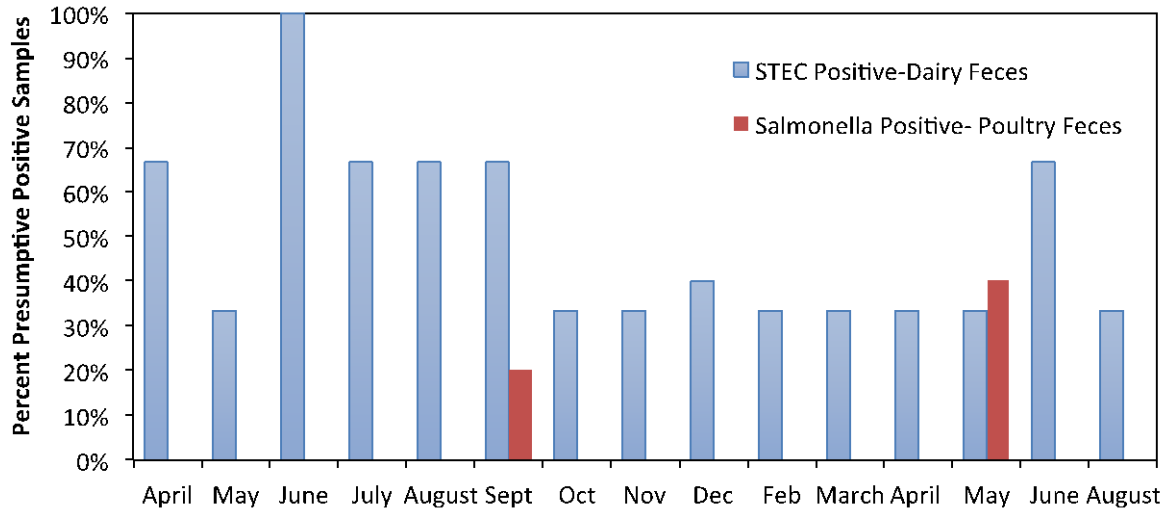
Bioaerosol sampling (n=140): From each location (32, 200 and 400 ft) within the field plots located in close proximity to dairy or poultry operations we collected a total of six samples at the defined distances (Appendix **Figure A2**). Air samples corresponded to 2 weeks of continuous (24/7) air collection at a rate of 10 L per min (total volume collected in 2 weeks =  $2.16 \times 10^6$  L of air). All air particles were collected on a fiberglass membrane capable of capturing up to PM1 particles (1µm). After 2 weeks of air capturing the fiberglass membrane was split into two equal sized sections and one was used for DNA extraction while the other was used for bacteria enumeration (Appendix **Figure A3**). In brief, bacteria enumeration was performed as follows: 7 ml of half strength BPW was added to the glass membrane, sonicated for 30 s and part of the supernatant was plated on half-strength TSA, ChromSTEC, ChromECC, and XLT4. The sonicated supernatant was later incubated for 24 h at 25°C, followed by 24-h incubation at 30°C. After this incubation process, 7 ml of UPB were added to each sample and incubated for 24 h at 37°C. After incubation the same procedure listed below for *Salmonella* and STEC was followed for the detection of enteric pathogens.

## Objective 1 Results

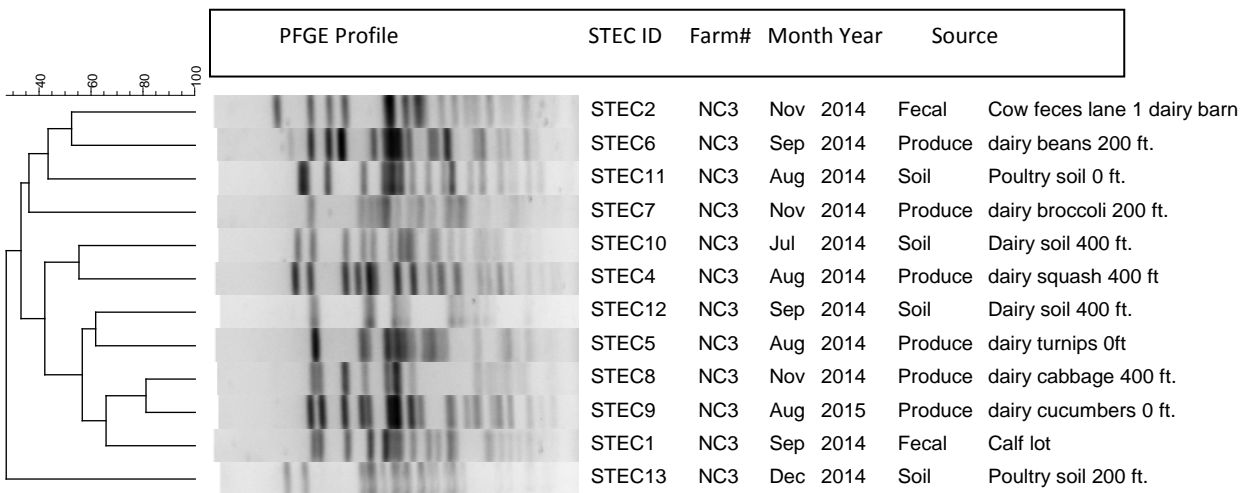
### Presence of *Salmonella* and STEC in Animal Operations, Agricultural Soils and Produce Samples

As expected, the prevalence of STEC in manure coming from different locations within the dairy farm was high (**Figure 2**). Nonetheless, the distribution and prevalence of these pathogens was not consistent over a 12-month period. Under NC weather conditions, the prevalence of STEC in dairy manure was higher during the transition from spring to summer months than during fall to winter. A large and heterogeneous population of STEC was observed from dairy manure collected from six different locations within the dairy unit. A total of 15 different STEC isolates were recovered from dairy manure and could not be clustered at high relatedness. **Figure 3** illustrates some of the diversity of these strains

and those recovered from soil and produce samples based on PFGE profiles. *S. Newport* was recovered on two occasions from poultry manure during the 12-month sampling series. These two events coincided with major cleanup events within the layer-hen house. In both instances the prevalence of *Salmonella* varied between 20–40% within the samples collected and had no clear distribution pattern.



**Figure 2.** Prevalence of STEC and *Salmonella* isolates extracted from dairy or layer-hen manure located in close proximity to diversified vegetable farming systems.

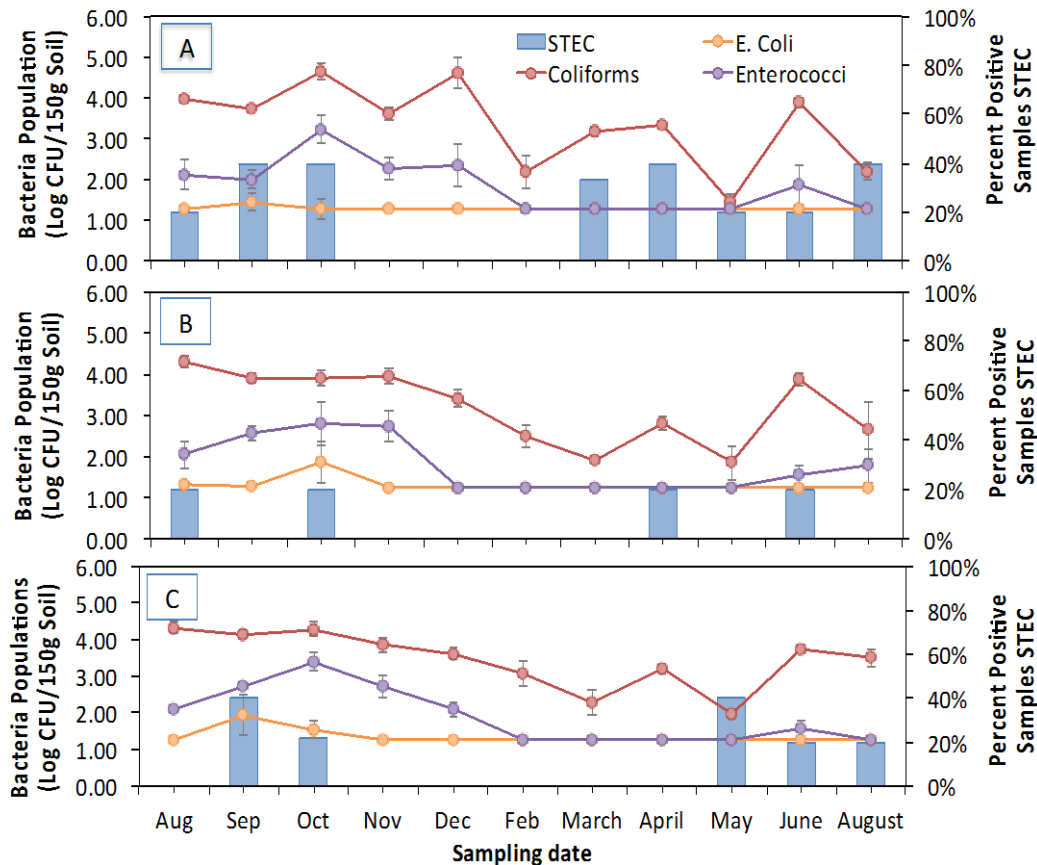


**Figure 3.** Dendrogram depicting genotypic similarities between STEC isolates coming from soil, feces and produce samples located in close proximity to dairy or layer-hen operations.

Dairy Unit – Diversified Vegetable Plots

The dairy unit consisted of about 150 heads of dairy cows spread across three main locations: milking area, calf barn and heifer barn. At these locations there was constant and continuous presence of animals and the wind pattern cut across this unit and pointed towards our diversified vegetable plots. These plots were managed using organic management practices, except for the fertilization regimes, and none received any application of dairy manure for a period of over 3 years. Two years before the establishment of our plots this field remained under native grass cover and no dairy run-off was detected in this period. Each of the 3 diversified vegetable plots (located at 32, 200 and 400 ft away from the

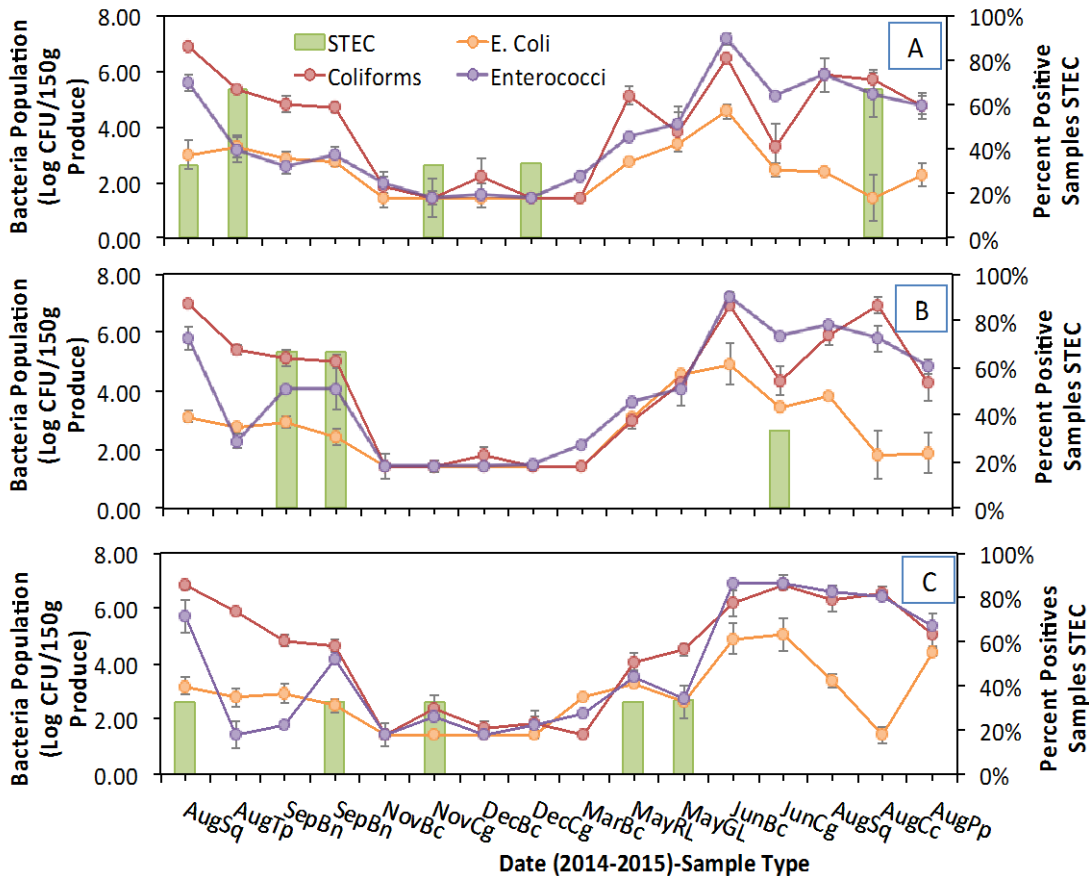
dairy unit) consisted of ten 30-inch beds where an array of vegetables was planted, including cover crops between rows. During each sampling event a total of 21 soil samples were collected and analyzed for the presence of STEC and indicator microorganisms. STEC was recovered on multiple occasions and locations within the 400-ft cropping system. A higher number of soil samples positive for STEC were recovered from samples taken at 32 ft away from the dairy unit than at 200 and 400 ft (Figure 4). The population of generic *E. coli* was between 1.2 and 2 log CFU/150 g of soil during the entire experiment, while the population of *Enterococci* varied between 1.2 and 3 log CFU/150 g of soil. As expected, total coliforms were present at concentrations between 2.5 and 5 log CFU/150 g of soil and remained consistent within and between locations. During the winter months all indicator microorganisms presented the lowest populations during the yearlong project.



**Figure 4.** Distribution and prevalence of STEC and indicator microorganisms isolated from soil samples collected (A) 32 ft, (B) 200 ft and (C) 400 ft away from dairy operations. Data represent averages of 21 samples per time point collected from different locations within each plot. Data were reported based on distance from the dairy unit instead of per crop and distance. Time 0 samples tested negative for *Salmonella* and STEC.

STEC microorganisms also were recovered from produce samples grown in close proximity to soil (squash, cucumbers, beans, lettuce and turnips) and those located several inches above the soil (cabbage, broccoli, peppers). No clear pattern in our ability to detect the presence of STEC was observed between crops in close proximity or several inches above soil and the weather conditions. All positive samples came from physiologically mature crops ready for harvest and from two independent harvest events (Figure 5). In most cases, similarities were at or above 50%, but no direct match was observed between feces, soil, or produce samples using PFGE. The presence of generic *E. coli*, coliforms and *Enterococci* was identical within and between locations. Coliforms and *Enterococci* had populations that ranged between 2 and 8 log CFU/150 g of produce, while for generic *E. coli* the populations were not higher

than 4.5 log CFU/150 g of produce. The highest populations for all three indicator microorganisms were recovered during the summer months and this coincided with the highest activity of cows within the dairy unit. The presence of positive STEC soil samples did not always coincide with the determination of positive STEC produce samples. For example, for the 400-ft distance during May through August 2015, all soil samples tested positive for STEC, but the crops grown during those months (lettuce, squash, cucumber, cabbage, broccoli, and peppers) tested negative for STEC. However, the 32- and 200-ft produce samples (squash and cabbage) were positive for STEC as were the soil samples collected from these two locations.

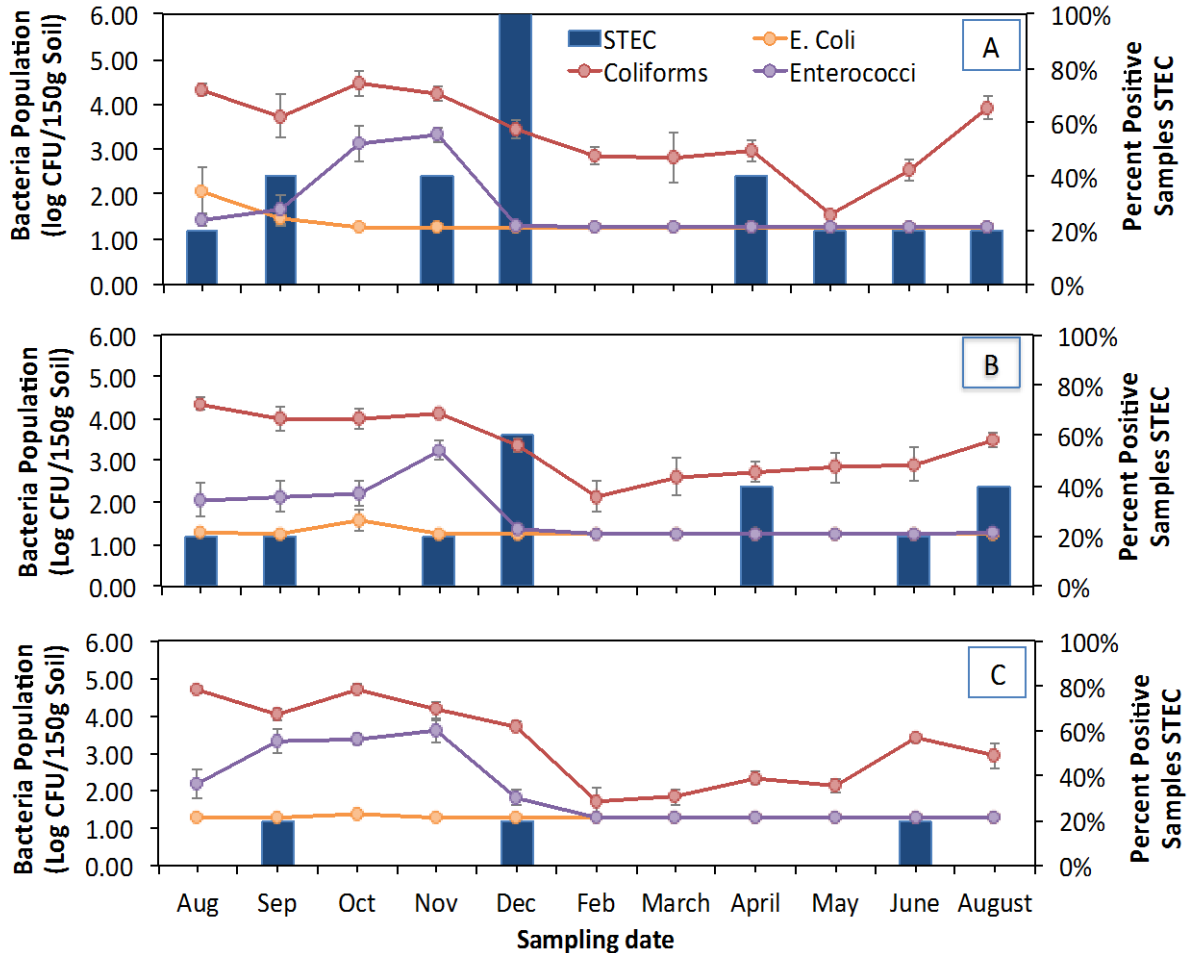


**Figure 5.** Distribution and prevalence of STEC and indicator microorganisms isolated from various produce samples collected (A) 32 ft, (B) 200 ft and (C) 400 ft away from dairy operations. Data represent averages of 18 samples per time point/produce collected from different locations within each plot. Each crop was sampled on two occasions and there was a 7-day interval between sampling events. Aug=August, Sep=September, Nov=November, Jun=June; Sq=squash, Tp=turnip, Bn=bean, Cg=cabbage, Bc=broccoli, RL= red lettuce, GL= green lettuce, Cc=cucumber, and Pp=peppers.

#### Poultry Unit – Diversified Vegetable Plots

The poultry unit diversified vegetable plots were established on land that had not been in cultivation for over 10 years and remained under native grass coverage for all these years until our experimental plots began. The same water source and fertilization regimes were used at this location and all produce was managed under organic practices except for nutrient applications. The layer-hen house had a population of 5,000 birds, all within their prime egg production stage. The vents of the house were directly facing our experimental plots and remained active throughout the entire yearlong project. Since the goal of this aspect of the project was mainly to address the potential transfer of *Salmonella spp.* from the poultry

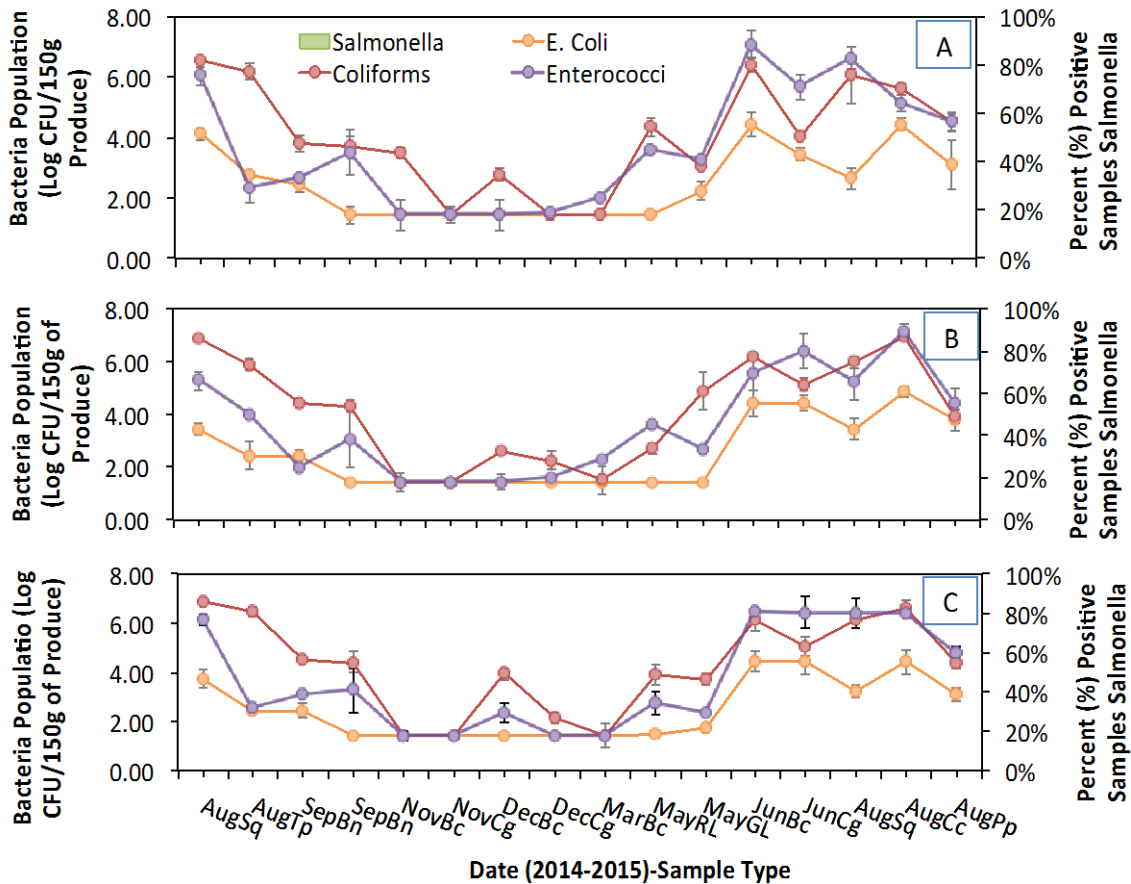
house to produce, we only tested vegetable samples for the presence of this pathogen whereas soil samples were also tested for STEC. We did not expect a high prevalence of STEC at this location; however, this was not the case (**Figure 6**). STEC was present within the established 400-ft growing area; however, a greater number of positive STEC soil samples and higher population levels were recovered at 32 ft away from the poultry house vents than at the 200- and 400-ft distances.



**Figure 6.** Distribution and prevalence of STEC and indicator microorganisms isolated from soil samples collected (A) 32 ft, (B) 200 ft and (C) 400 ft away from poultry operations. Data represent averages of 21 samples per time point collected from different locations within each plot. Data were reported based on distance from the dairy unit instead of per crop and distance. *Salmonella* was not recovered from any of the soil samples collected from these locations. Time 0 samples tested negative for *Salmonella* and STEC.

The populations of indicator microorganisms were also identical between growing locations. As expected, coliforms also were present at higher populations than generic *E. coli* and *Enterococci* and were similar to those determined from soil samples collected at the dairy unit (**Figure 4** and **6**). The winter months also had the lowest populations of all indicator microorganisms. *Salmonella* spp. were not recovered from any of the soil samples collected at all three locations. The genotypic characteristics of some of these strains isolated from poultry soil samples were compared to produce and soil samples collected near the dairy unit (**Figure 3**). Results suggest little to no genotypic similarity between STEC microorganisms isolated within poultry or dairy units, suggesting intrinsic characteristics specific to each animal operation and reinforcing the idea of a potential transfer specific to each location. Further, all soil samples collected just after plowing and preparing the fields near the dairy or poultry unit

resulted in zero positives for any of the pathogens, suggesting that contamination happened after this disturbance event. Produce samples were not tested for the presence of STEC, but all samples tested negative for the presence of *Salmonella* spp. (**Figure 7**). The highest populations of all indicator microorganisms were also observed during the summer months; however, the populations of generic *E. coli* were similar to the other two indicator microorganisms and this trend was not observed with the population of this same microorganism when extracted from produce samples grown near the dairy unit.



**Figure 7.** Distribution and prevalence of *Salmonella* and indicator microorganisms isolated from various produce samples collected (A) 32 ft, (B) 200 ft and (C) 400 ft away from poultry operations. Data represent averages of 18 samples per time point/produce collected from different locations within each plot. Each crop was sampled on two occasions and there was a 7-day interval between sampling events. Aug=August, Sep=September, Nov=November, Jun=June; Sq=squash, Tp=turnip, Bn=bean, Cg=cabbage, Bc=broccoli, RL= red lettuce, GL= green lettuce, Cc=cucumber, and Pp=peppers.

### Water Analysis

During the duration of the project we collected a total of 274 L of water, with half obtained from each of the two growing locations. The population of generic *E. coli*, coliforms and *Enterococci* remained below the microbiological criteria established by the EPA recreational water standard. Several spike events were observed during 6 out of the 12 sampling events; however, none had a significant negative impact in the microbial quality of the water used for irrigation. All samples tested negative for the presence of *Salmonella* spp. and STEC (Appendix **Figure A4**). This water was only delivered via drip irrigation for all crops, since transplant seedlings were used to establish each crop. In North Carolina during spring, summer and fall, the growing season's rain provides additional water to the crops, which in general precludes the need to use sprinkler irrigation. All water samples were collected by the same individual

and at the same outlet of the irrigation system within the dairy and poultry operations. Under the new produce rules established this water would be considered acceptable to be used in direct contact with the edible portion of the crop, although this was never the case during the trials. The same water was used to supply the fertilizer requirements for each crop.

### Air Analysis

Despite our ability to sample air continuously for 2 weeks, which corresponded to  $2.16 \times 10^6$  L of air in this time interval, *Salmonella* or STEC were not recovered from any of the 140 samples collected from the dairy and poultry field plots. The presence of generic *E. coli* and total coliforms was established on 40% of the samples collected during one year; however, these indicator microorganisms were recovered only after enrichment (Appendix **Figure A3**). DNA was extracted from all samples and used to sequence the bacterial 16S and the fungal ITS1 regions. Note: At the time of report submission we are awaiting results from the genome center before beginning the microbial community analysis of samples.

## **Objective 2 Results**

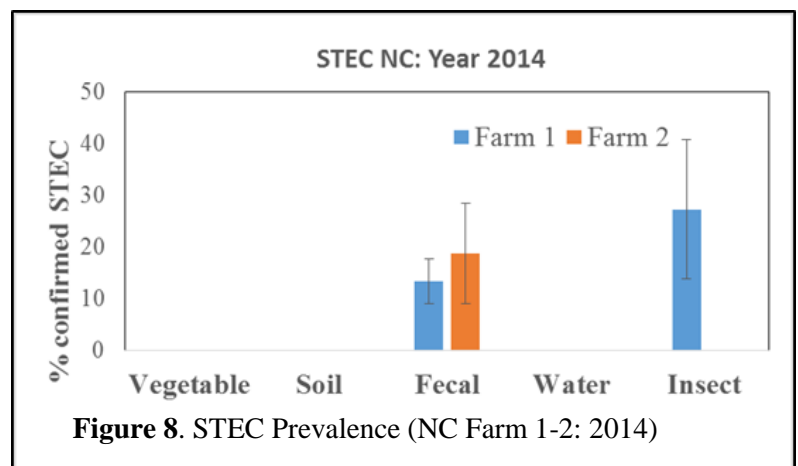
### North Carolina Year 1

A total of 289 samples, including produce (n=110), soil (n=122), poultry feces (n=60), water (n=3), and insects (n=11), were collected from Farm 1 that was sampled twice in the Piedmont region of NC. We were able to recruit a second sustainable farm late in the year and this farm was sampled only once in December 2014. From Farm 2 we collected a total of 72 samples, including produce (n=40), soil (n=16), and dairy cattle feces (n=16). Date and location of each sample's collection event were recorded. Samples were

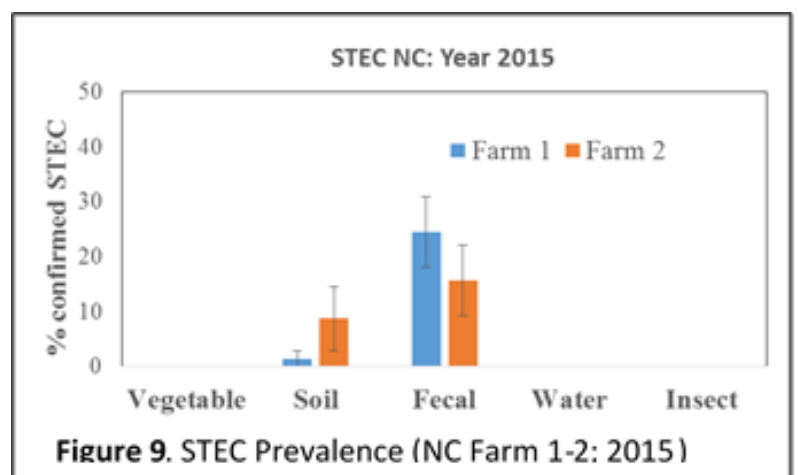
processed to isolate *Salmonella* and STEC. Any putative positive samples were collected from the plates and stored until further analysis. A consent form was signed and received from the farm owner to proceed forward with further testing of the presumptive positive samples. None of the samples from the two farms tested positive for *Salmonella* upon further analysis. However, from Farm 1 we isolated STEC from eight fecal (13.3%) and three fly (27%) samples (**Figure 8**). From Farm 2 we isolated STEC from three fecal samples (18.75%). None of the STEC isolated from Farms 1 or 2 tested positive for *E. coli* O157:H7.

### North Carolina Year 2

The same two NC farms were sampled repeatedly (3 times each) between May–September 2015. From Farm 1, we collected 181 samples comprising vegetables (n=52), soil (n=71), poultry feces (n=45), insects (n=10), and water (n=3). From Farm 2 we collected an additional 150 samples, including vegetables (n=47), soil (n=23), cattle feces (n=32), insects (n=15), and water (n=1).



**Figure 8.** STEC Prevalence (NC Farm 1-2: 2014)



**Figure 9.** STEC Prevalence (NC Farm 1-2: 2015)

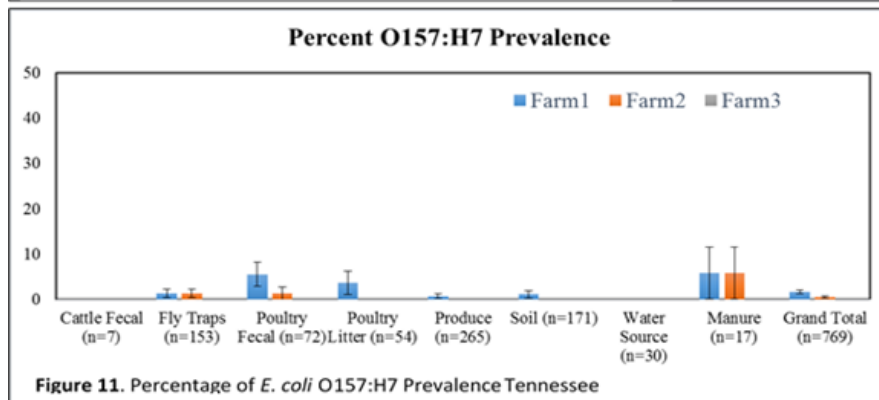
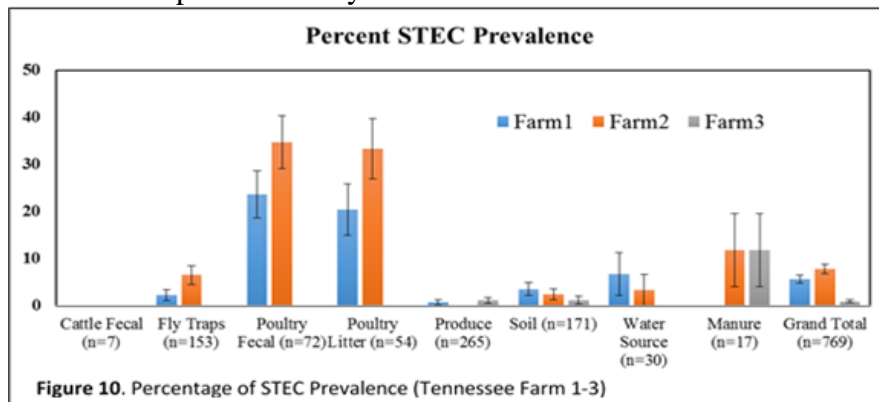
Samples were processed individually to isolate *Salmonella* and STEC. Any presumptive positive cultures collected from agar plates were prepared and stored until further analysis. No *Salmonella*-positive samples were detected from these two farms in year 2 of sampling. From Farm 1 we isolated STEC from 11 fecal samples (24.4%) and detected a single STEC-positive sample from soil (1.4%). From Farm 2, STEC was isolated from five fecal (15.6%) and from two soil (8.7%) samples (**Figure 9**). None of the STEC isolates were O157:H7 as determined by PCR testing.

### Tennessee Year 1

A total of 769 of samples, including cattle feces (n=7), insects (fly traps; n=153), poultry feces (n=72), poultry litter (54), fresh produce (n=265), soil (n=171), water (n=30), and manure (n=17), were collected from three sustainable TN farms in 2014. Each farm was sampled three times during 2014. The date and location at which these samples were collected on each farm was recorded. We isolated and confirmed a total of 110, 17, and 62 isolates of STEC, *E. coli* O157:H7, and *Salmonella*, respectively.

### STEC and O157:H7 Prevalence:

STEC was isolated predominantly from samples originating from Farm 1 (n=43) and Farm 2 (n=60) compared with Farm 3 (n=7) during the year (**Figure 10**). The number of STEC-positive samples was significantly higher from Farm 2 than from Farm 1 ( $P = 0.01$ ). STEC prevalence in both Farms 1 and 2 was highest among the poultry samples, including fecal and litter. Fresh produce samples from Farm 1 (1.1%) and Farm 3 (6.5%) tested positive for STEC; the produce samples that tested positive included melons, cucumbers and beans. There was no significant difference between STEC prevalence detected in fly traps ( $P = 0.31$ ) and water source ( $P = 0.25$ ) between Farm 1 and 2. Based on PCR testing, a total of 17 STEC isolates (13.3%) were serotyped O157:H7, which were recovered from various sample types, including produce (beans), soil (bean field), poultry litter, poultry feces, and fly traps (**Figure 11**). The serotype O157:H7 was predominantly isolated from Farm 1.



**Salmonella** Prevalence:

A total of 40 isolates of *Salmonella* were isolated from the multiple sampling conducted at the three farms. *Salmonella* was isolated predominantly from Farm 2 (n=50; 80.6%) followed by Farm 1 (n=11; 17.7%) and Farm 3 (n=1; 1.6%). The sample types that tested positive included poultry feces, poultry litter, soil, and manure; none of the produce samples tested positive (**Figure 12**). The predominant serotype detected was 4,5,12:i:- (45%), followed by Enteritidis (17.5%) and Schwarzengrund (15%).

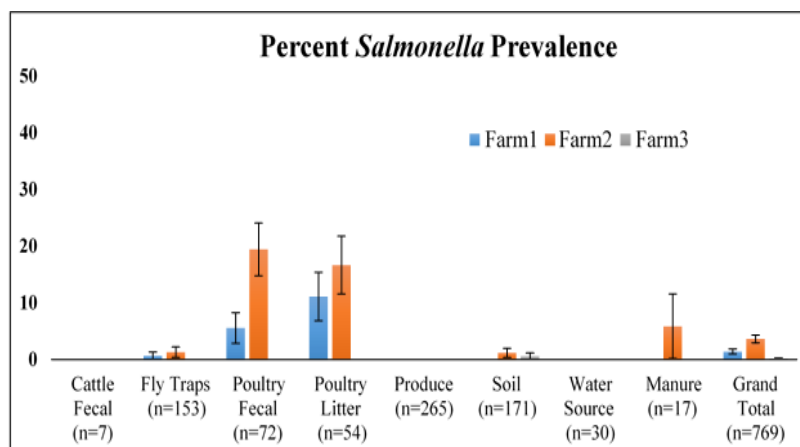


Figure 12. Percentage of *Salmonella* Prevalence (Tennessee Farm 1-3)

**Tennessee Year 2**

Two farms were sampled in TN in 2015 and each farm was sampled three times. We collected 298 samples (fecal, litter, produce, soil, water, and flies) from the two farms (**Table 1 and 2**). A total of 91 STEC isolates were obtained from Farm 1 (n=27) and Farm 2 (n=64). All the presumptive STEC and O157:H7 isolates were confirmed by PCR. Serotype O157 was detected from two samples (water and cucumber) from Farm 1 but none from Farm 2. *Salmonella* (n=22) was isolated in Farm 2 from fecal samples (n=8), litter samples (n=10), and fly traps (n=4); the predominant serotype detected was Schwarzengrund (59%), followed by Kentucky (13.6%) and Typhimurium (13.6%).

Farm 1 (N=151)	Fecal (N=27)			Produce (N=55)			Soil (N= 39)			Water (N=12)			Fly Traps (N=18)		
	May	June	July	May	June	July	May	June	July	May	June	July	May	June	July
<i>Salmonella</i> (n)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STEC (n)	4	3	7	0	2	7	2	0	0	2	0	0	0	0	0
STEC O157 (n)	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0

Farm 2* (N=147)	Fecal (N=32)			Litter (N=36)			Soil (N= 26)			Water (N=10)			Fly Traps (N=42)		
	May	June	July	May	June	July	May	June	July	May	June	July	May	June	July
<i>Salmonella</i> (n)	6	1	1	6	2	2	0	0	0	0	0	0	0	1	3
STEC (n)	12	5	8	12	7	10	0	0	0	2	1	1	1	2	3
STEC O157 (n)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

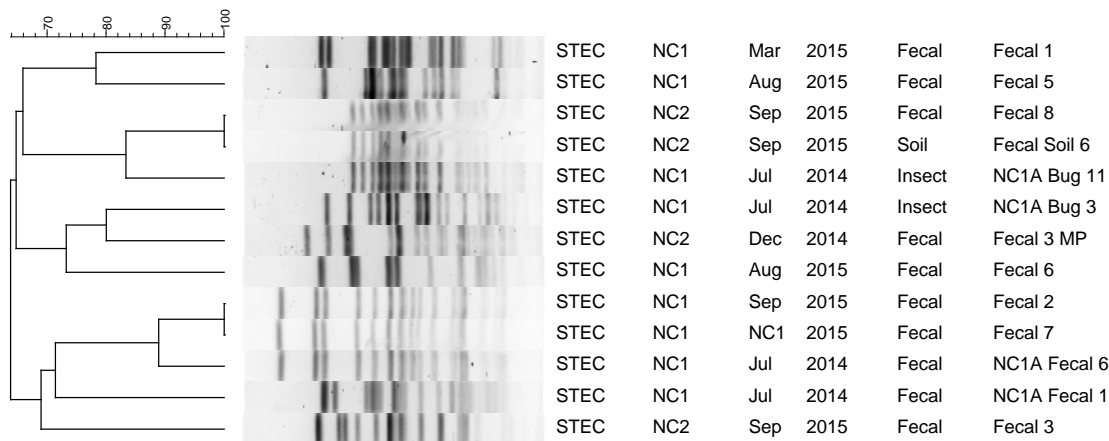
**Table 1 and 2.** Total number of samples and isolates positive for STEC and STEC O157, and confirmed *Salmonella*, obtained during the 2015 sample period. \*No crops were produced during this period in TN. N = total number of samples collected; n= number of samples positive.

The detection of similar serotypes in flies and the chicken litter and fecal samples confirm the role of flies in the persistence of *Salmonella* on chicken farms. As reported before, because chickens eat the flies, they may become colonized and shed the pathogen and contaminates the litter becoming in an important source of cross-contamination if directly applied to the fields or if composting does not kill the *Salmonella*.

### Pulse field gel electrophoresis (PFGE)

#### North Carolina:

We randomly selected 13 STEC isolates (year 2014: 5 isolates; year 2015: 8 isolates) from the two sustainable farms in NC for genotypic characterization using PFGE (**Figure 13**). These isolates were recovered from different sample types including feces, soil, and insects. Based on fingerprint profile and dendrogram analysis, the STEC isolate from the soil sample (fecal soil 6) shared an identical PFGE pattern with the fecal sample (fecal 8) in the same area. We also detected fecal samples with 100% relatedness (fecal 2 and 7). At approximately 82–87% similarity, fecal samples from year 2014 and 2015 were clustered in the same group (2015: fecal 2, 7; and 2014: fecal 6). Moreover, the isolates from insect, fecal and soil samples had a close relationship (fecal 8, soil 6, and insect 11).

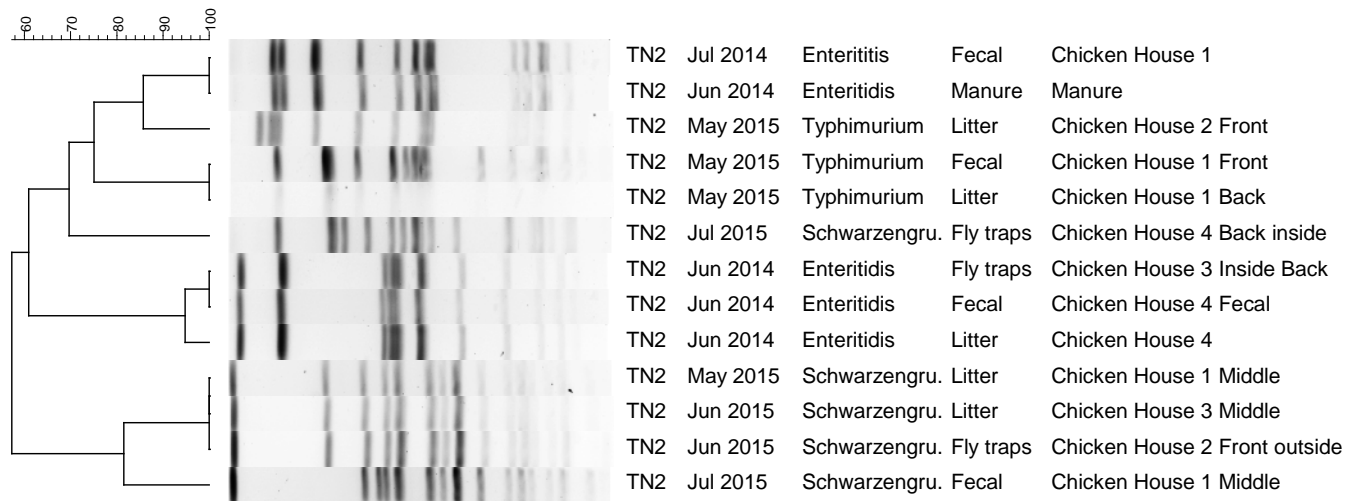


**Figure 13.** Dendrogram showing genotypic similarity of STEC isolates from North Carolina Farms 1 and 2.

#### Tennessee:

The PFGE profiles of STEC isolated from Tennessee sustainable farms are shown in Appendix **Figure A5**. Both STEC (n=9) and O157 (n=4) from Tennessee Farm 1 were selected for genotypic characterization. Two of the O157 isolates were unique and did not group with other STEC isolates. Two STEC isolates from produce (tomato and cucumber) collected at the same time exhibited 100% genotypic similarity. Different kinds of samples are in each cluster including soil, feces, water, and produce. However, two selected O157 isolates from Tennessee were unable to be typed and could not be analyzed by PFGE after repeated trials.

A total of 13 *Salmonella* isolates representing three serotypes including Enteritidis (n=5), Typhimurium (n=3) and Schwarzengrund (n=5) from Tennessee Farm 2 were selected for PFGE analysis. The same serotype isolates were group together in the dendrogram, and isolates from several parts of the farm had a clonal relationship with isolates from different sampling times (**Figure 14**).



**Figure 14.** Dendrogram showing genotypic similarity of *Salmonella* isolates from Tennessee Farm 2.

### Outcomes and Accomplishments

1. STEC was recovered from soil and produce samples at a 400-foot distance between the animal sources and produce field. This was independent of the size of animal operation.
2. *Salmonella* was isolated from soil and animal fecal samples, but not from produce samples in the study from the two states.
3. *Salmonella* isolation was restricted to the Tennessee farms and was not isolated from any produce sample at either of the North Carolina or Tennessee farms.
4. Pathogen presence on the farm was associated with farm management rather than distribution in air.
5. STEC and O157:H7 were isolated from both animal sources and produce.
6. Our results strongly suggest that there needs to be a reassessment of the proposed 400-foot minimum buffer zone distance between animal operations and the location of fresh produce fields. Based on this study, this distance does not seem sufficient to prevent the transmission of pathogens from animal source to produce. We suggest conducting quantitative studies at varying buffer zone distances to determine the adequate buffer zones to prevent pathogen transmission and the establishment/assessment of other physical barriers to potentially reduce pathogen transfer.
7. Soil and insects were found to be positive for STEC, O157:H7 and *Salmonella*, which clearly highlights their potential for pathogen transmission to fresh produce.
8. The study has enabled us to form a consortium of fresh produce farmers in North Carolina who are now more eager and willing to collaborate with us on larger projects.

### Summary of Findings and Recommendations

The focus was on pathogen transfer from animal operations to diversified vegetable farming practices, however, our findings are not limited to the sustainable agriculture environment since other cropping systems, including standard organic production, conventional farming practices, and biodynamic approaches, will also pose the risk of pathogen transfer when in close proximity to animal operations. Under these scenarios the size of animal operation, proximity to produce fields, and cultural practices seem to impact pathogen transfer to produce and these parameters are irrespective of farming approaches. Consequently, whether farmers are

dealing with fruits, vegetables, tree nuts, horticulture, nursery crops, or others included within the definition of "specialty crops," our findings and potential approaches to reduce pathogen transfer could benefit any of them.

For our second objective, we collected farm samples, including produce, animal and the environmental, from commercial sustainable farms in North Carolina and Tennessee. There were clear distinctions between the outcomes based on geographic locations of these farms in the two different states. Overall, *Salmonella* was isolated from multiple samples originating from commercial sustainable farms only in Tennessee and not in North Carolina. None of the fresh produce samples from the farms in either of the two states tested positive for *Salmonella*. In Tennessee, STEC was isolated from fresh produce and animal and environmental sources as well as flies, while in North Carolina only the fecal and soil samples tested positive. Our results strongly suggest that there needs to be a reassessment of the proposed 400-foot minimum buffer zone distance between animal operations and the location of fresh produce fields. Based on our study, this distance does not seem sufficient to prevent the transmission of pathogens from animal sources to produce. We suggest conducting quantitative studies at varying buffer zone distances to determine the adequate buffer zones to prevent pathogen transmission and the establishment/assessment of other physical barriers to potentially reduce pathogen transfer.

Our recommendation for future projects, based on what we learned through this study, is to include funds to offset the financial burden to grower participants. The study has enabled us to form a consortium of fresh produce farmers in North Carolina who are now more eager and willing to collaborate with us on larger projects. We found that working closely with county-based extension staff on identifying and recruiting grower participants to be extremely valuable.

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## **APPENDICES**

### **Publications and Presentations**

1. Thakur S. (2015). Food safety risks at the Fresh Produce-Animal Interface: Identifying pathogen sources and their movement on diversified farms. CPS Produce Research Symposium, Atlanta, Georgia, June 23-24.
2. Carter R, Massel M, Gunter C, Thakur S, and Gutierrez-Rodriguez E. (2015). Food safety risks at the Fresh Produce–Animal Interface: Identifying pathogen sources and their movement on diversified farms. International Association for Food Protection Annual Meeting, Portland, Oregon.
3. Thakur S, Gutierrez E, Gunter C, Chapman B and Hanning I. (2014). Food safety risks at the fresh produce–animal interface: identifying pathogen sources and their movement on diversified farms. CPS Produce Research Symposium, Newport Beach, California.

#### **Workshop:**

Food Safety on Livestock and Produce Mixed Production Farms. (2015). Carolina Farm Stewardship Association Sustainable Agriculture Conference, Durham, North Carolina.

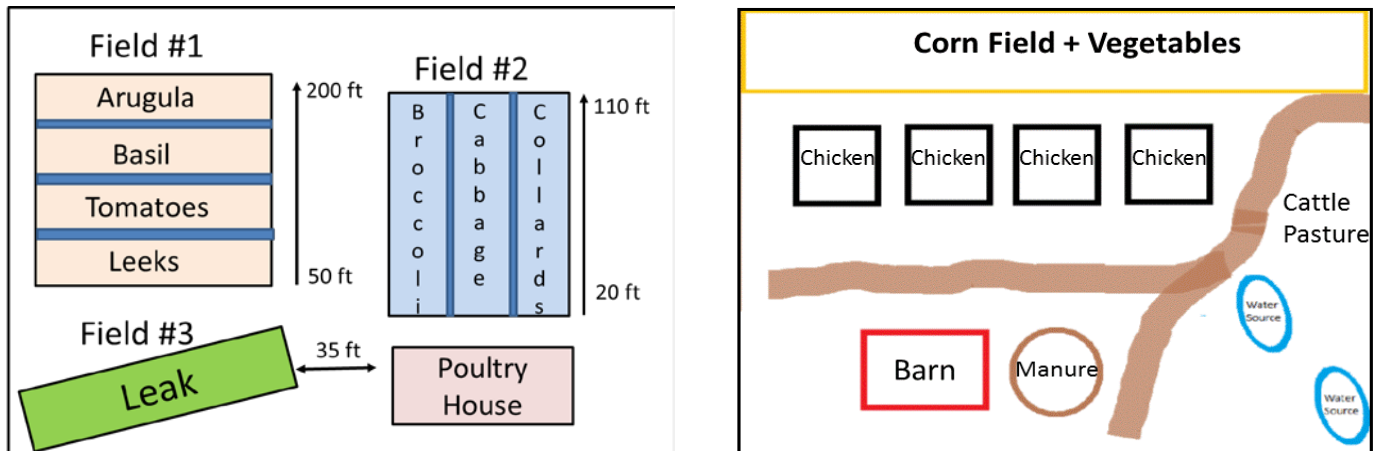
#### **Symposium:**

A Real-world Conversation about Food Safety and Microbial Quality of Sustainable Diversified Farming Systems. [Submitted] International Association for Food Protection Annual Meeting, St. Louis, Missouri, July 31–August 3, 2016.

### **Budget Summary**

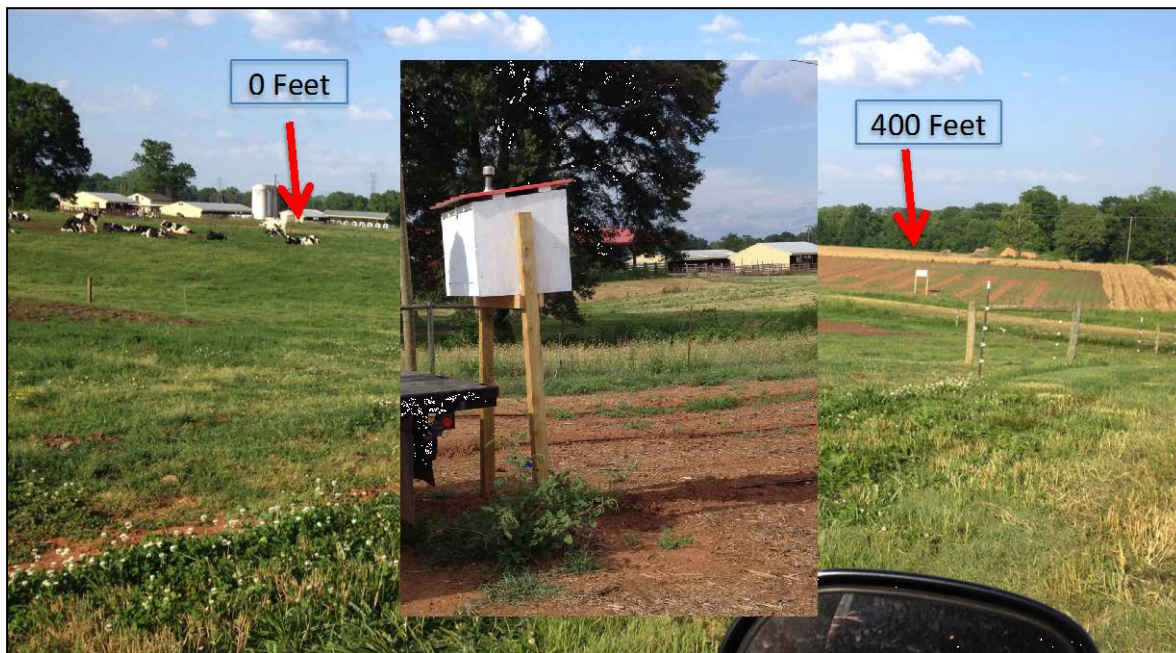
There is a remaining balance of ~\$34,000. Of this total, ~\$2,400 will be used for travel to the CPS Research Symposium in June 2016 and to cover publication costs; ~\$30,000 will not be used.

### Appendix Figures

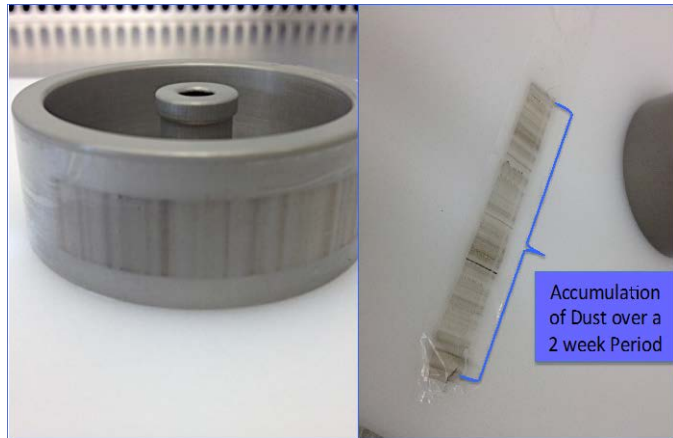
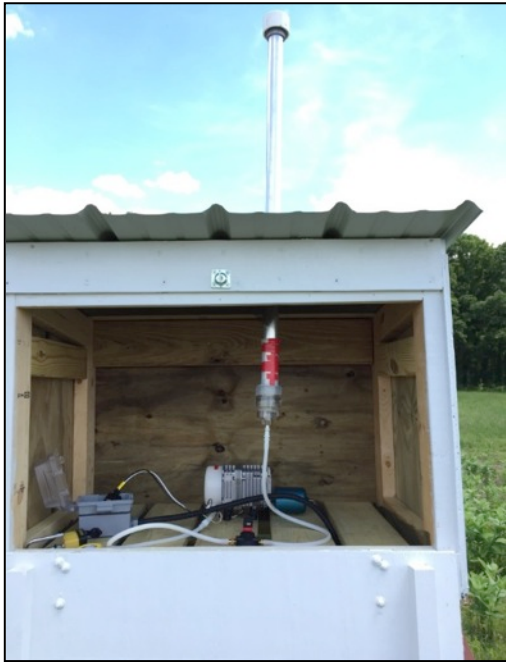


**Figure A1.** Farm layout for sustainable farm sampled in NC (left) and TN (right).

(Note: Field #3 contained leeks.)

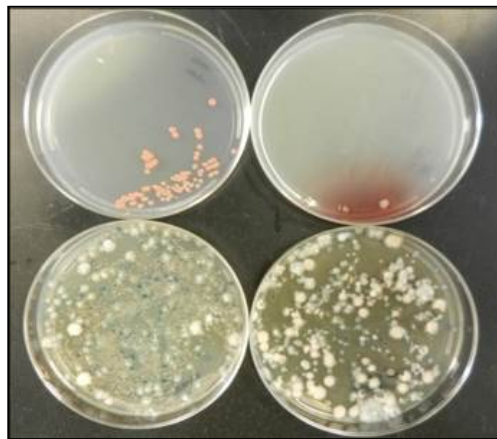


**Figure A2.** Location of air filters in fresh produce fields relative to the animal source.



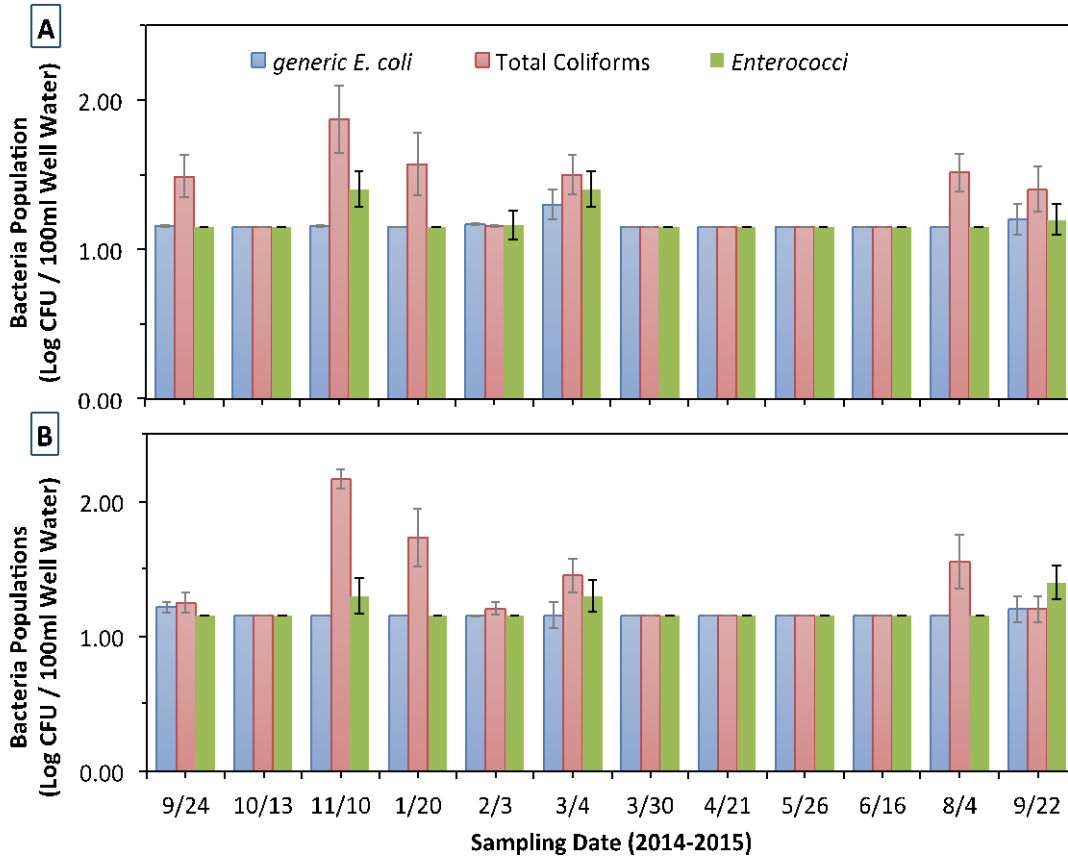
**Figure A3.** Air filter locations on the field (left). Air filter drum (above) and the accumulation of dust on the filter over a 2-week period. ChromSTEC plates (below) before and after enrichment.

### ChromSTEC Plates

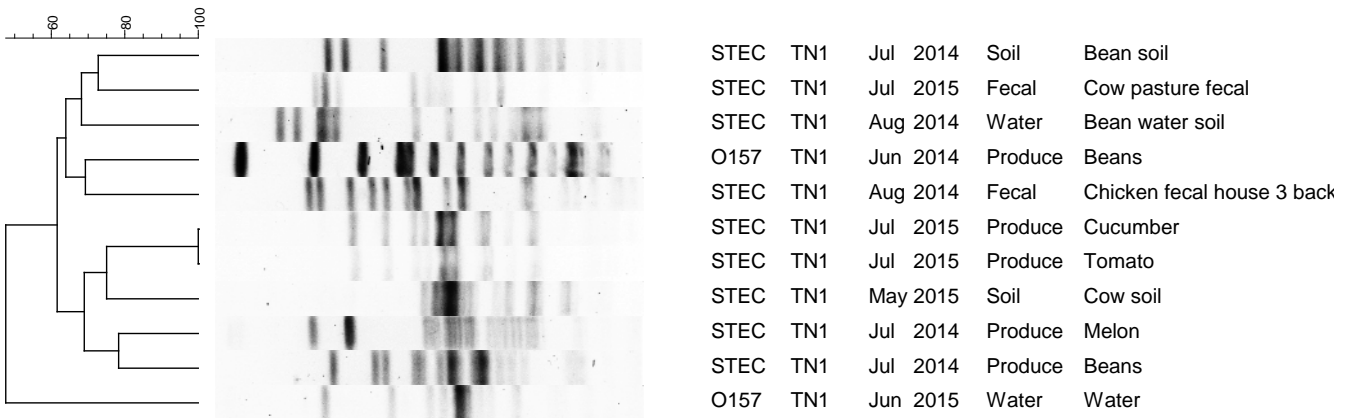


Before  
Enrichment

After  
Enrichment



**Figure A4.** Microbial water quality parameters of well water used for irrigation in both diversified vegetable fields location in close proximity to dairy (A) and poultry (B) operations. Data represent averages of six 1-gallon samples collected at the outlet of the irrigation system. All samples were negative for STEC or *Salmonella* spp.



**Figure A5.** Dendrogram showing genotypic similarity of STEC isolates from Tennessee Farm 1.

**Suggestions to CPS**

There was one major challenge that we faced in both states (NC and TN), which was the inability to recruit farms or rather the unwillingness of farm owners to participate in our study. One of the major issues in recruiting farms was our inability to compensate the farmers for their losses incurred due to sampling. In the future we would like to propose to the CPS that they allow a minor part of the funds towards compensating the producers for their potential loss due to sampling. We are not recommending cash payments, but rather compensation given out in the form of supplies like seeds, compost and other farm supplies. This will tremendously help us to recruit more farms for the study. We recommend that the advisory board to CPS consider our request to continue work on sustainable farming systems, as these farmers need assistance and guidance from the produce industry to deal with on-farm challenges that may compromise the safety of their product.