CENTER for PRODUCE SAFETY

CPS 2017 RFP
FINAL PROJECT REPORT

Project Title
Establishment of vegetative buffer zones to reduce the risk of STEC and *Salmonella* transmission from animal operations to fresh produce on co-managed farms

Project Period
January 1, 2018 – December 31, 2019 (extended to February 28, 2020)

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Objectives

1. *Develop a fast-growing and cost-effective vegetative buffer zone (VBZ) that growers in the southeastern United States can implement within a 1–2 year period to prevent movement of STEC and Salmonella from animal production areas (APAs) to fresh produce fields.*
2. *Evaluate the fate and potential movement of STEC and Salmonella before, during and after the establishment of a VBZ between APAs and fresh produce fields.*
3. *Determine the potential of the VBZ to reintroduce STEC and Salmonella contamination into adjacent produce fields.*

Funding for this project provided by the Center for Produce Safety through:
CDFA SCBGP grant# 17-0275-051-SC

FINAL REPORT

Abstract

Co-managing livestock and fresh produce continues to be linked to high-risk potential for pathogen transmission and contamination, while land and water resources continue to dwindle in many of our fruit and vegetable production areas. The experimental and rationale framework of this proposal is based on a previously completed research project in which we targeted the movement of pathogens from animal production areas (APAs) to produce fields within 30–400 ft buffer zone distances. In our recently published 2-year CPS study (Glaize et al., 2020), we clearly showed the transmission of *Escherichia coli* from livestock to fresh produce in sustainable farms where animals are, by design, in close proximity to fresh produce. The results indicate that (a) the Leafy Green Marketing Agreement (LGMA) suggested buffer zone distance of 400 ft (122 m) between APAs and the edge of a crop field, and (b) the 30 ft (9 m) buffer zone for grazing lands or domestic animals and the edge of the crop field may not be appropriate. Consequently, further studies are needed to revise this distance and to identify functional and cost-effective barriers that can reduce pathogen transfer to produce fields without negatively impacting land availability and wildlife habitat. Additionally, as U.S. farmers are complying with the FSMA Produce Safety rule and, as part of their environmental risk assessment and potential corrective actions, they will need to implement practices that can reduce the risk of pathogen transfer and contamination from APAs to adjacent produce fields.

Currently there are few alternatives to reduce this transfer from APAs to produce other than moving field operations to another location. Based on the results of the previous research and new preliminary information, this project proposed to follow a hurdle approach to address two of the most important and elusive questions around co-management: 1) what is the risk associated with proximity of vegetative areas as a potential source of human pathogen contamination to fresh produce?, and 2) can these areas actually serve as barriers or sinks for pathogen transmission to produce even in the presence of APAs? Answers to these two questions are key to the sustainability of different farming practices across the globe and specifically in the southeastern United States, where small to medium-size animal producers, vegetative and/or riparian areas, and woodlands are in close proximity to produce fields.

Background

Even following major advances and improvements in the field of microbial food safety, foodborne diseases associated with consumption of contaminated fresh produce remain among the leading public health concerns. The interaction of typically host enteric-adapted bacterial pathogens with fresh produce grown in fields is complex and can occur by multiple pathways, including: 1) application of inadequately composted or raw animal manure; 2) run-off from nearby food animal farms to produce fields; 3) use of surface waters for overhead irrigation and for frost protection; and 4) pathogen transmission via insects and domesticated and wild animals. These interactions are more prominent in co-managed or sustainable farms where animal operations are, by design, near fresh produce, and growers frequently move between the two production environments. Sustainable farming systems operate to maximize resource utilization with the recognition that green or animal waste can be composted reclaimed and used in another farming practice to provide adequate nutrients to crops and soil health; however, this practice could increase the risk of produce contamination and foodborne illnesses if not treated and used following standard practices.

Recent co-management recommendations do focus on wind-based movement of pathogens. However, few studies have looked at the wind dispersal of foodborne pathogens and potential dust abatement. Instead, these studies have looked at some basic principles of air dust/odor/pathogen movement with limited information specific to fruit and vegetable production. Our hypothesis is that the movement of human pathogens from APAs to produce fields located within 400 ft (122 m) from these animal operations is primarily driven by wind dispersal, and by establishing a vegetative buffer zone (VBZ) between animal operation and field could reduce and potentially control movement of pathogens from APAs into adjacent produce fields.

Research Methods and Results

Objective 1. Develop a fast-growing and cost-effective vegetative buffer zone (VBZ) that growers in the southeastern U.S. can implement within a 1–2 year period to prevent movement of STEC and Salmonella from animal production areas (APAs) to fresh produce fields.

Establishment of VBZ: The zones were established in April 2016 between produce fields and the respective dairy and poultry operations at the Piedmont Research Station (NC). Each buffer zone is 100 ft long and 30 ft wide (30 m long and 9 m wide) (**Figure 1**). The 100 ft length was divided into rows in 3 blocks: 1) one row of rapid growing hardwood trees, 2) two rows of evergreen trees and shrubs, and 3) a non-manicured grass strip. The selection of plant materials was based on three primary factors: rapid growth rate, availability, and cost effectiveness. After reaching full growth capacity of each species, the average final height of each VBZ block was 1) 12 ft (3.7 m), 2) 9 ft (2.7 m) and 6.8 ft (2.1 m), and (3) 1.7 ft (53 cm).

Objective 2. Evaluate the fate and potential movement of STEC and Salmonella before, during, and after the establishment of a VBZ between APAs and fresh produce fields.

The first sampling was performed in April 2018, when the team collected manure, air, barrier plants, and soil in order to establish a baseline of prevalence of STEC, *Salmonella*, and indicator microorganisms at each location. The dairy and poultry farms were sampled at 14 separate time points during 2018 (8 visits) and 2019 (6 visits) to collect soil, produce and manure samples; air and plant barrier samples were collected more frequently in 2018 (10 visits) and 2019 (7 visits). For Objective 2, 1,133 environmental samples were collected during the 2-year study (this total does not include the samples collected during the challenge study of Objective 3).

Presence of *Salmonella* and STEC in bio-aerosols, barrier plants, manure, produce, soil: Bio-aerosol, barrier plant, manure, produce, and soil samples were collected from the sites at selected times, and then transported (at 4°C) back to the lab. Samples were subsequently processed according to the sample type to determine the presence or absence of presumptive *Salmonella* or STEC; presumptive colonies were isolated. The colony polymerase chain reaction (PCR) was used to confirm species identification of isolates by the presence of virulence genes *inv*A for *Salmonella* or *stx*1 and *stx*2 for STEC, and to verify the 16S rRNA sequence for *E. coli*. Sampling and processing details are provided below.

Bio-aerosol sampling: Air samples were collected at 32, 200, and 400 ft (10, 61, and 122 m) away from the VBZ. A total of 119 air samples were collected from these locations (**Figure 2** shows an air sampler at the 32-ft location) over the course of the hurdle approach; 7 air samples (1 – control, 3 – dairy, 3 – poultry) were collected at each sampling trip. Seven impactors with membrane filters were used to collect bio-aerosol samples and filter air at a rate of 10 L/min; particles larger than 1 µm were captured in the glass-based filter for up to 2 weeks. Filters were

transported back to the lab for analysis. A sterile razor blade was used to cut the air filter in half; one half of the filter was frozen at -80°C and the other half was cut again in half. These two quarter-sections of the filter were then mixed with 7 mL of 1/2X buffered peptone water (BPW) with pyruvate (1 mL/L), massaged, incubated at 25°C for 24 H, and incubated again at 30°C for another 24 H. After incubation, 7 mL of universal pre-enrichment broth (UPB) was added to the enrichment and incubated for 24 H at 35°C.

Salmonella: One quarter of the filter and 6 mL of UPB enrichment were transferred into 14 mL of Rappaport-Vassiliadis (RV) broth and incubated at 35°C for 24 H. After incubation, 100 µL of enrichment was plated onto xylose lysine tergitol 4 (XLT4) media and incubated overnight at 35°C. Three black colonies from each plate were then further isolated, transferred into 20% glycerol stock, and frozen at -80°C until PCR confirmation.

STEC: One quarter of the filter and 6 mL of UPB enrichment were transferred into 14 mL of mEHEC broth and incubated at 35°C for 24 H. After incubation, 100 µL of enrichment was plated onto CHROMagar STEC media and incubated overnight at 35°C. Mauve colonies present on CHROMagar STEC were further isolated, transferred into 20% glycerol stock, and frozen at -80°C until PCR confirmation.

Barrier plant sampling: A total of 204 barrier plant samples were collected from the VBZ locations over the course of the hurdle approach; 12 barrier plant samples (6 – cattle, 6 – poultry) were collected at each sampling trip. The weight of each sample was recorded and potassium phosphate (KPO_4; 2:1 ratio) was added to each bag. After sonication for 30 min, 90 mL of solution was split evenly between two 50 mL conical tubes. Both tubes were spun at 2,000 rpm for 10 min at 4°C. After disposal of the supernatant, one tube was frozen at -20°C and the pellet in the other tube was re-suspended with 10 mL of KPO_4; 10 mL of the KPO_4 solution was added to 90 mL of 2X UPB and incubated overnight at 35°C.

Salmonella: After incubation, 5 mL of UPB enrichment was added to 45 mL of tetrathionate broth base (TBB) and incubated at 42°C for 6 H. Next, 10 mL TBB enrichment was then transferred to 90 mL of mBroth and incubated at 35°C overnight. After incubation, 10 µL of mBroth enrichment was plated onto XLT4+PCNB (pentachloronitrobenzene) and incubated overnight at 35°C. Three black colonies from each plate were then further isolated, transferred into 20% glycerol stock, and frozen at -80°C until PCR confirmation.

STEC: After incubation, 10 mL of UPB enrichment was added to 90 mL of mBroth and incubated at 35°C overnight. Next, 10 µL of mEHEC enrichment was plated onto CHROMagar STEC+PCNB and incubated overnight at 35°C. Mauve colonies present on CHROMagar STEC+PCNB were further isolated, transferred into 20% glycerol stock, and frozen at -80°C until PCR confirmation.

Manure sampling: At the beginning of the study, 6 fecal samples each were collected from the two APAs, for a total of 12 samples at each sampling time. However, in August 2018 the poultry house was depopulated, which reduced the total number of fecal samples collected at each visit to 6 (from the dairy operation only). Overall, 114 fecal samples (30 – poultry, 84 – dairy) were collected. Fecal samples, ~150 g each, were collected with sterile plastic scoops from freshly excreted feces of cattle livestock and layer-hen manure.

Salmonella: In brief, 10 g of raw manure was mixed with 90 mL of BPW and incubated at 35°C overnight. After incubation, 100 µL of BPW enrichment was transferred into 9.9 mL of RV broth and incubated at 42°C overnight. Then 10 µL of the RV enrichment was streaked onto XLT4 media and incubated overnight. Three black colonies from each plate were then further isolated, transferred into 20% glycerol stock, and frozen at -80°C until PCR confirmation.

STEC: In brief, 20 g of raw manure was mixed with 180 mL of UPB and incubated overnight at 35°C. Next, 10 mL of UPB enrichment was transferred into 90 mL of mEHEC and incubated overnight at 35°C. Then 10 µL of the mEHEC enrichment was streaked onto

CHROMagar STEC and incubated overnight. Mauve colonies present on CHROMagar STEC were further isolated, transferred into 20% glycerol stock, and frozen at -80°C until PCR confirmation.

Produce sampling: Produce samples (lettuce or tomato) were collected from the produce fields at 32, 200, and 400 ft from the APAs. Samples were taken from every other plant in each row. An effort was made to harvest the tomatoes at different stages of maturation. If the plant did not have any tomatoes, the leaves were collected instead. Overall, 336 produce samples were collected. Each produce sample weighing 150 g was placed into a Whirl-Pak bag along with 300 mL of 2X UPB and massaged for 1 min, and then the sample was sonicated for 30 min. The supernatant was transferred to another bag and incubated overnight at 35°C.

Salmonella: In brief, 90 mL of TBB was placed in a Whirl-Pak bag and then 10 mL of the enriched produce sample was added and the mixture was incubated for 6 H at 42°C. Next, 20 mL from the enrichment was added to 180 mL of mBroth and then incubated overnight at 35°C. Lastly the sample was streaked onto XLT4 agar. The plates were incubated for 36 H at 35°C and then examined for colonies. Colonies that were presumptive *Salmonella* were selected with a 10 µL loop for further isolation and then confirmed through PCR.

STEC: In brief, 90 mL of mEHEC was placed in a 1-L Whirl-Pak bag and then 10 mL of the enriched sample was added and the mixture was incubated for 18–24 H at 35°C. After incubation the enrichment was plated onto CHROMagar STEC and incubated for 24 H at 35°C. Mauve colonies present on CHROMagar STEC were further isolated for confirmation by PCR.

Soil sampling: A sterile scoop was used to collect soil samples on each side of every selected row in the produce fields. Samples were taken at every fourth plant and placed into separate 1 L Whirl-Pak bags, for processing the following day. Overall, 360 soil samples were collected. Each soil sample weighing 150 g was mixed with 150 mL of 0.02M sodium phosphate + Tween, and then the bag was massaged for 1 minute. Next, the entire supernatant was filtered to a new bag, 150 mL of 2X UPB was added, and then the bag was incubated overnight at 35°C.

Salmonella: In brief, 90 mL of TBB was placed in a Whirl-Pak bag and then 10 mL of the enriched soil sample was added and the mixture was incubated for 6 H at 42°C. Next, 20 mL from the enrichment was added to 180 mL of mBroth, then incubated overnight at 35°C. The sample was streaked onto XLT4 agar + PCNB, which is selective for *Salmonella*; plates were incubated for 36 H at 35°C and then examined for colonies. Those presumptive for *Salmonella* were selected with a 10 µL loop for further isolation, and then confirmed through PCR.

STEC: In brief, 90 mL of mEHEC was placed in a Whirl-Pak bag and then 10 mL of the enriched sample was added and the mixture was incubated for 18–24 H at 35°C. After incubation the enrichment was plated onto CHROMagar STEC + PCNB and incubated for 24 H at 35°C. Mauve colonies present on CHROMagar STEC + PCNB were further isolated for confirmation by PCR.

Objective 3. Determine the potential of the VBZ to reintroduce STEC and Salmonella contamination into adjacent produce fields.

The goals of the challenge study were 1) to determine the “barrier capacity” of the buffer zone under a worst-case scenario event, and 2) to determine the impact of VBZ removal on transfer of the inoculated strains and naturally occurring STEC and *Salmonella* into fresh produce. The first part of the challenge study was completed between October and November 2019, once each VBZ reached full maturity.

Inoculation and sampling of dairy and poultry VBZs: Lab strains of avirulent *E. coli* O157:H7 and *Salmonella enterica* Typhimurium with resistance to rifampicin, up to 80 ppm, were used to

separately inoculate 100 lb of chalk (inoculum carrier) at 7 log CFU/g of chalk. Initially, the inoculated chalk was divided into six bins and allowed to dry naturally for 3 weeks; this resulted in each bin drying at a different rate, which may have affected the results of the first trial. To address this issue, the team decided to perform a second round of the inoculation study. For the second trial, the inoculated chalk was split into six bins, and two large fans were used to help dry the chalk inoculum at the same rate for approximately 2 weeks. The poultry VBZ was challenged with the *Salmonella enterica* Typhimurium strain, and the dairy cattle VBZ was challenged with the *E. coli* O157:H7 strain. A leaf blower and ramp were used to transfer the chalk inoculum onto each VBZ (**Figure 3**). On the day of inoculation (day 0), environmental samples (air, soil, produce, and barrier) were collected before and 1 hour after inoculation. Samples were collected on day 0, day 7, and day 14 at 32, 200, and 400 ft away from each VBZ. Initially, the sampling was also to occur on day 30, but this was not done since there was no recovery of the inoculated *E. coli* or *Salmonella* strains by day 14. Recovery and confirmation of the inoculated avirulent *E. coli* O157:H7 and *Salmonella* strains from samples were performed following the same procedures as described above. It is important to note that rifampicin was added to all media except for TBB to select for the lab strain avirulent *Salmonella enterica* Typhimurium.

This experiment was repeated twice due to low recovery rates of avirulent *E. coli* O157:H7 and *Salmonella* after the first inoculation trial. A total of 403 environmental samples (trial 1, n=268; trial 2, n=135) were collected. Repetitive element PCR (rep-PCR) was done to compare the lab avirulent *E. coli* O157:H7 and *Salmonella enterica* Typhimurium strains to isolates recovered during the challenge study.

Due to the low recovery rates of avirulent *E. coli* O157:H7 and *Salmonella enterica* Typhimurium from both inoculation trials, and how late in the season these experiments took place, which resulted in poor canopy coverage, the team decided not to remove the VBZs. Therefore the second part of this challenge objective, to determine the impact of VBZ removal on pathogen transfer, was not carried out. Given that the inoculum was positive for all strains 24 H before application, the low recovery rates clearly indicated that the dissemination of the inoculum in the air and the presence of the buffer zone helped in significantly reducing the dose, which is a positive outcome of our efforts.

Outcomes and Accomplishments

Objective 1
A fast-growing and cost-effective vegetative buffer zone (VBZ) was achieved, and reached full growth capacity of each plant species in June 2019. The final heights of the VBZ components in the three blocks were as follows: block 1 – 12 ft /3.7 m (poplar trees); block 2 – 9 ft /2.7 m (loblolly pines), and 6.8 ft /2.1 m (green giant arborvitae); and block 3 – 1.7 ft /53 cm (rye grass). Each VBZ was located within 46 ft (14 m) of the animal operation (dairy or poultry) and 32 ft (~10 m) from the first rows of the respective produce field (**Figures 1** and **2**).

Objective 2
The dairy and poultry farms were sampled up to 10 times in 2018 and up to 7 times in 2019 to collect the various environmental samples. A total of 1,133 environmental samples, including produce (n= 336), soil (n= 360), air (n= 119), barrier plants (n= 204), and manure (n= 114), were collected during the project period. Of these environmental samples, only 7 samples were confirmed positive for *Salmonella* (**Figure 4**), and 82 samples tested positive for STEC (n=53) and generic *E. coli* (n=29) from various sources on the dairy and poultry farms (**Figure 5**).

In total, 19 *Salmonella* isolates (confirmed by PCR) were obtained from the barrier plants, produce, and soil of the poultry farm (n=10) as well as from dairy cattle raw manure (n=9) (**Figure 6**). *Salmonella* isolates from produce (n=3) were obtained from one lettuce sample collected at the poultry farm in April 2019 (at a distance of 32 feet from the VBZ – see Figure 9), and the isolates from the VBZ (n=3) were obtained from one barrier plant sample collected in May 2018.

In total, 101 STEC isolates and 55 *E. coli* isolates were obtained from the various environmental samples (**Figure 7**). Almost all the isolates were from samples collected in April and May 2019.

More environmental samples positive for STEC and *E. coli* were collected from the dairy cattle farm than from the poultry farm (**Figure 8**). It is notable how many samples collected 200 and 400 ft away from the dairy and poultry VBZs were positive for STEC and *E. coli*. Of the four confirmed *Salmonella*-positive samples collected at the poultry farm, the lettuce sample and one soil sample were collected 32 ft from the VBZ, and one soil sample was collected at 400 ft from the VBZ (**Figure 9**); the positive soil sample at 400 ft was collected in 2018 before the poultry house was depopulated. (The one positive barrier plant sample is not shown in Figure 9.)

Objective 3

Barrier challenge studies were attempted twice in 2019: trial 1 between October 14–31 (3 sampling times), and trial 2 between November 18–29 (2 sampling times). Trials were performed out of season at a time when the deciduous trees in each VBZ had lost their leaves and the main vegetative barrier was established by the pine trees and grass strip (Figure 3). The late timing was largely the result of challenges that occurred in the project involving key personnel and the university. Also, some equipment used in the microbiome evaluations was not available for over 1.5 months, which further delayed the completion of several activities.

Results from the two trials, despite the low barrier availability, showed little transfer of the inoculated strains to the produce fields. Further, the inoculated strains were not recovered at most post inoculation events, despite the inoculum concentration at 6 log CFU/g when tested 24 hours before inoculation. The low recovery of the inoculated strains from the fields was likely due in part to dilution of the inoculum over a large area as well as the barrier impact of the VBZ (Figure 3). The poultry farm VBZ was challenged with a *Salmonella enterica* Typhimurium strain and the dairy cattle farm VBZ was challenged with an *E. coli* O157:H7 strain. Environmental samples (air, barrier plants, produce, and soil) were collected before and after inoculation of the VBZ; a total of 403 environmental samples (trial 1, n=268; trial 2, n=135) were collected. During trial 1, 13 *E. coli* O157 isolates and 2 *Salmonella* isolates were obtained from the samples; during trial 2, 5 *E. coli* isolates were obtained from the samples. Of these isolates, only one *E. coli* O157:H7 isolate (from a soil sample in trial 2) had a similar banding pattern to the rifampicin-resistant avirulent *E. coli* O157:H7 lab strain used in the inoculum. Details of the trial results are provided below.

Trial 1 results (**Figure 10**): At day 0, rifampicin-resistant *Salmonella* was recovered from soil samples (n=2) collected 200 ft away from the poultry VBZ before the inoculation of the barrier. However, the results of rep-PCR revealed that the *Salmonella* recovered from poultry soil before inoculation was different to that of the lab *Salmonella enterica* Typhimurium strain. None of the environmental samples collected before or after the inoculation of the dairy cattle VBZ tested positive for rifampicin-resistant *E. coli* O157:H7. At day 7, none of the environmental samples collected from the poultry farm tested positive for rifampicin-resistant *Salmonella* after enrichment. However, 13 samples collected from the dairy cattle farm were positive for rifampicin-resistant *E. coli* O157:H7; most of these positives were soil samples (n=11) collected at 32 ft (n=4), 200 ft (n=4), and 400 ft (n=3) from the VBZ, and one was a lettuce sample

collected at 200 ft from the VBZ. However, of the *E. coli* isolates recovered, none had a similar banding pattern to the rifampicin-resistant avirulent *E. coli* O157:H7 lab strain. At day 14, all environmental samples collected from both farms were negative for *E. coli* and *Salmonella*.

Trial 2 results (**Figure 11**): At day 0, one air sample collected at 200 ft from the dairy farm VBZ before the second inoculation was positive for rifampicin-resistant *E. coli* O157:H7. After inoculation, three soil samples collected at 32 ft (n=1) and 200 ft (n=2) from the dairy farm VBZ were positive for rifampicin-resistant *E. coli* O157:H7. The results of rep-PCR revealed that only one of the *E. coli* isolates recovered (from soil collected at 200 ft, row 2, from the dairy VBZ) had a similar banding pattern to the rifampicin-resistant avirulent *E. coli* O157:H7 lab strain. At day 7, no environmental samples collected were positive for rifampicin-resistant *E. coli* O157:H7 or rifampicin-resistant *Salmonella enterica* Typhimurium. Also, no bacteria were recovered from either the *E. coli* O157:H7 chalk inoculum or the *Salmonella enterica* Typhimurium chalk inoculum. This finding, along with only minimal recovery of rifampicin-resistant *E. coli* O157:H7 on the first day after inoculation, led to the decision to stop the second trial and no additional sampling was conducted.

Summary of Findings and Recommendations

The data from whole genome sequencing (to be completed) will reveal if the STEC and *Salmonella* isolates sourced from dairy cattle manure (n=20) and poultry manure (n=1, STEC) are similar to the STEC and *Salmonella* isolates sourced from the dairy cattle and poultry farm air, barrier, soil, and produce samples collected between 2018–2019. Preliminary data analysis suggests that the contamination by STEC and *Salmonella* did not come from the feed operations but rather that the contamination was already present in the soil. Despite the depopulation of the poultry house, 22 environmental samples from the poultry farm tested positive for *E. coli* and STEC (n=18) or *Salmonella* (n=4). This result suggests the need for more effective bioremediation practices to decontaminate agricultural soils.

Preliminary data analysis from the challenge study suggests that a vegetative buffer zone could be used to mitigate the transmission of STEC and *Salmonella* from APAs to adjacent produce fields. Despite the low barrier coverage at the time of inoculation with *E. coli* O157:H7 and *Salmonella enterica* Typhimurium inoculums at 6 log CFU/g, rep-PCR analysis showed that only one of the positive *E. coli* O157:H7 isolates recovered had a similar banding pattern to the rifampicin-resistant avirulent *E. coli* O157:H7 lab strain used in the challenge inoculum. This result suggests that farmers can use VBZs to aid produce safety on co-managed farms or sustainable farms where animal operations are, by design, near fresh produce.

APPENDICES

Publications and Presentations

There are no publications from this project prepared to date.

Presentations

Ayanna Glaize, Morgan Young, Eduardo Gutierrez-Rodriguez, and Siddhartha Thakur. "*The Use of Vegetative Buffer Zones to Reduce the Risk of Salmonella Transmission from Animal Operations to Fresh Produce*," [poster] presented at Triangle Global Health Consortium, Durham, NC, October 2019.

Ayanna Glaize, Morgan Young, Eduardo Gutierrez-Rodriguez, and Siddhartha Thakur. "*The Use of Vegetative Buffer Zones to Reduce the Risk of Salmonella Transmission from Animal Operations to Fresh Produce*," presented at CVM Research Forum, Raleigh, NC, August 2019.

Ayanna Glaize, Morgan Young, Eduardo Gutierrez-Rodriguez, and Siddhartha Thakur. *"The Use of Riparian Buffer Zones to Reduce the Risk of Salmonella Transmission from Animal Operations to Fresh Produce*," [T1-08] presented at International Association for Food Protection Annual Meeting, Louisville, KY, July 2019.

Morgan Young, Ayanna Glaize, Christopher Gunter, Siddhartha Thakur, Eduardo Gutierrez-Rodriguez. "*Establishment of Vegetation Buffer Zone Areas to Reduce Transfer of Enteric Pathogens from Animal Operations to Fresh Produce*," [T1-07] presented at International Association for Food Protection Annual Meeting, Louisville, KY, July 2019.

Budget Summary

The total funds awarded to this project were $378,924, and the majority of funds were spent.

Reference cited

Glaize A, Gutierrez-Rodriguez E, Hanning I, Díaz-Sánchez S, Gunter C, van Vliet AHM, Watson W and Thakur S. (2020). Transmission of antimicrobial resistant non-O157 *Escherichia coli* at the interface of animal-fresh produce in sustainable production environments. *International Journal of Food Microbiology* 319:108472. https://doi.org/10.1016/j.ijfoodmicro.2019.108472.

Figures (see below)

Figures 1–11

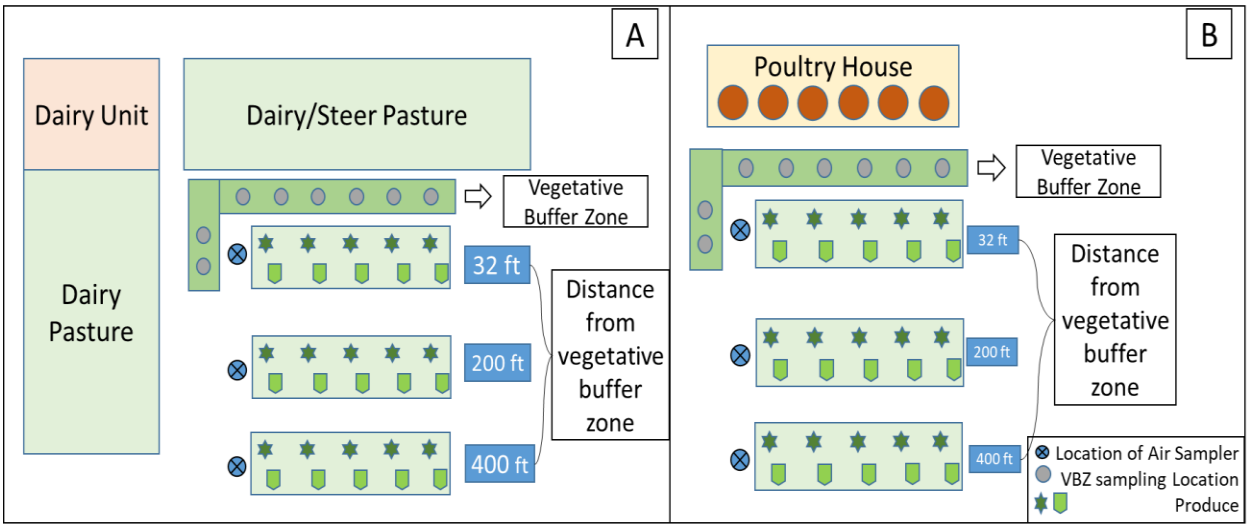

Figure 1 – Study design: Schematic and overall layout of the VBZ established at the Dairy (A) and Poultry (B) operations located at the Piedmont Research Station. Schematic depicts the location of each animal operation in reference to the location of each VBZ and produce field, and the locations of the crops.

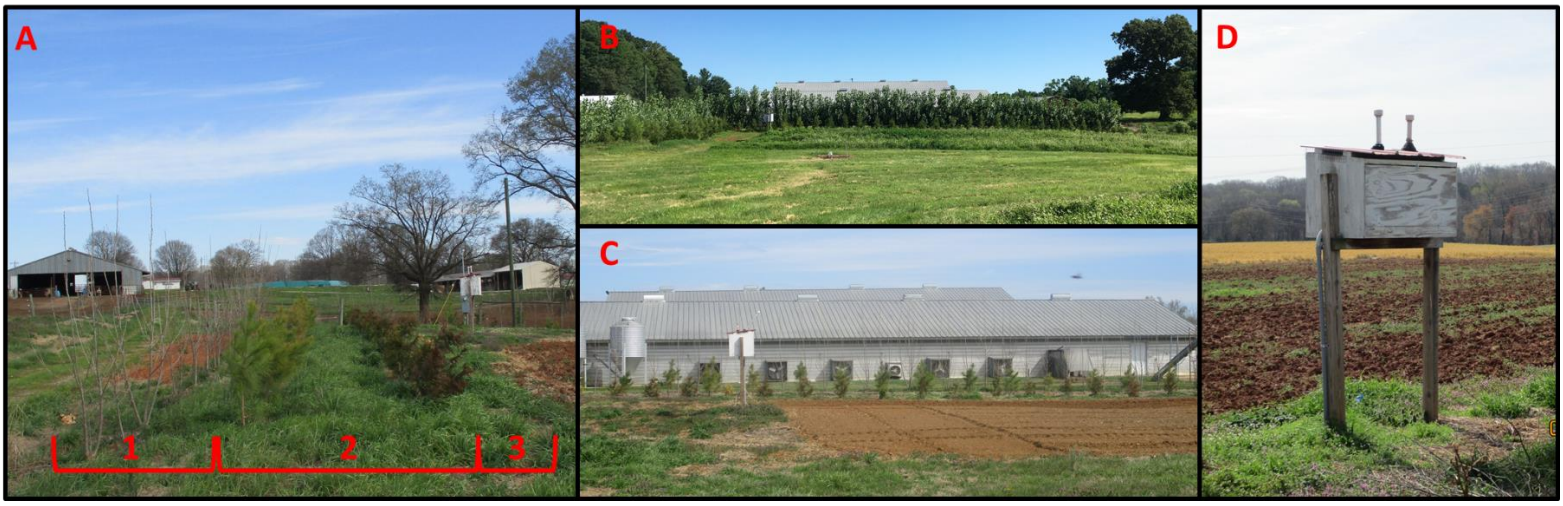

Figure 2 – Barrier visualization: A) The VBZ located at the dairy cattle farm in April 2018: Block 1 – hardwood trees, Block 2 – 2 rows of evergreen trees and shrubs, Block 3 – grass strip and pollinator plants. B) The fully established VBZ located at the poultry farm in June 2019. C) The growing VBZ located at the poultry farm in April 2018. D) Air sampler at 32 ft distance from the VBZ located at the dairy cattle farm.

Figure 3 – Challenge study inoculation: A) Ramp setup for inoculation of poultry farm VBZ in trial 2. B) Chalk inoculum application to the dairy cattle farm VBZ during trial 2. C) Poplar tree row in dairy cattle farm VBZ after inoculation in trial 1.

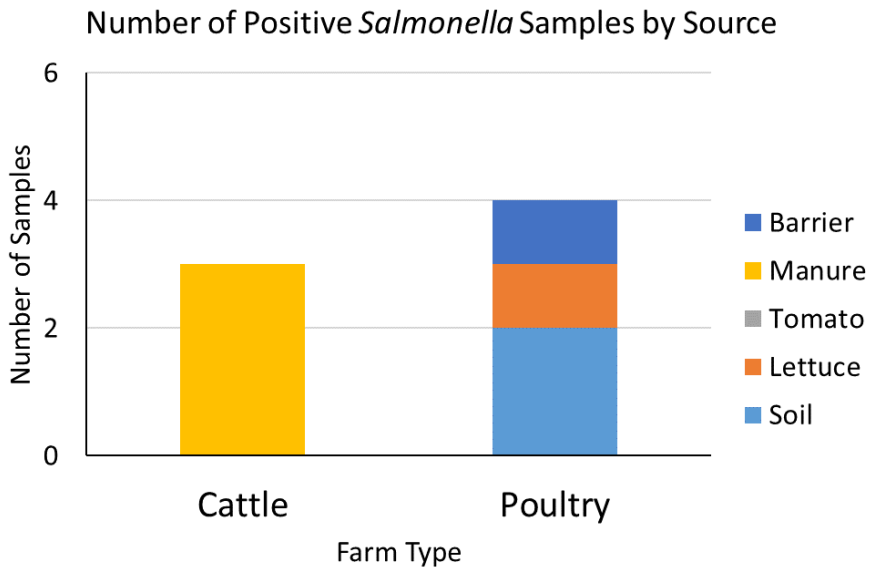

Figure 4 – Number of *Salmonella*-positive samples recovered by farm source. *Salmonella* was recovered from samples of dairy cattle manure (n=3), poultry farm soil (n=2), poultry farm lettuce (n=1), and the poultry farm VBZ plants (n=1).

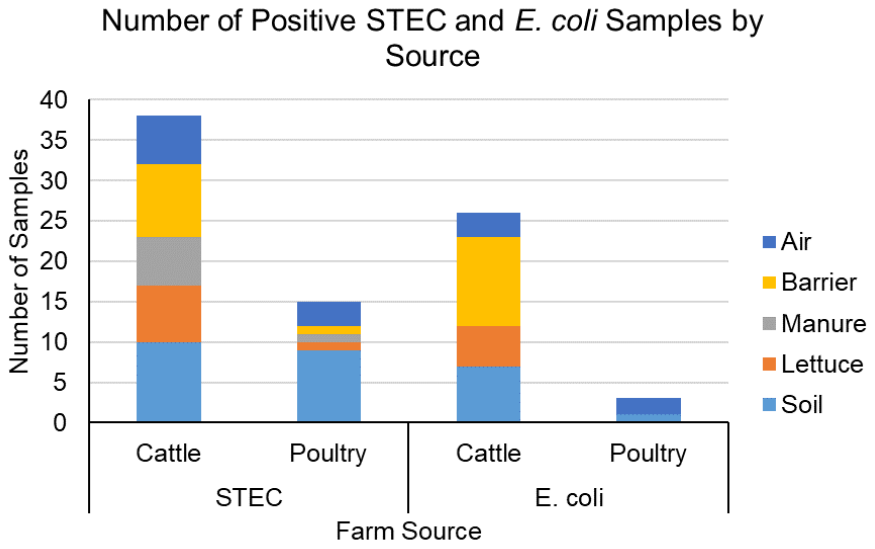

Figure 5 – **Number of positive STEC and *E. coli* samples recovered by farm source.** STEC was recovered from 1) dairy cattle farm samples: soil (n=10), lettuce (n=7), manure (n=6), barrier plants (n=9), and air (n=6); and 2) poultry farm samples: soil (n=9), lettuce (n=1), manure (n=1), barrier plants (n=1), and air (n=3). Generic *E. coli* was recovered from 1) dairy cattle farm samples: soil (n=7), lettuce (n=5), barrier plants (n=11), and air (n=3); and 2) poultry farm samples: soil (n=1) and air (n=2).

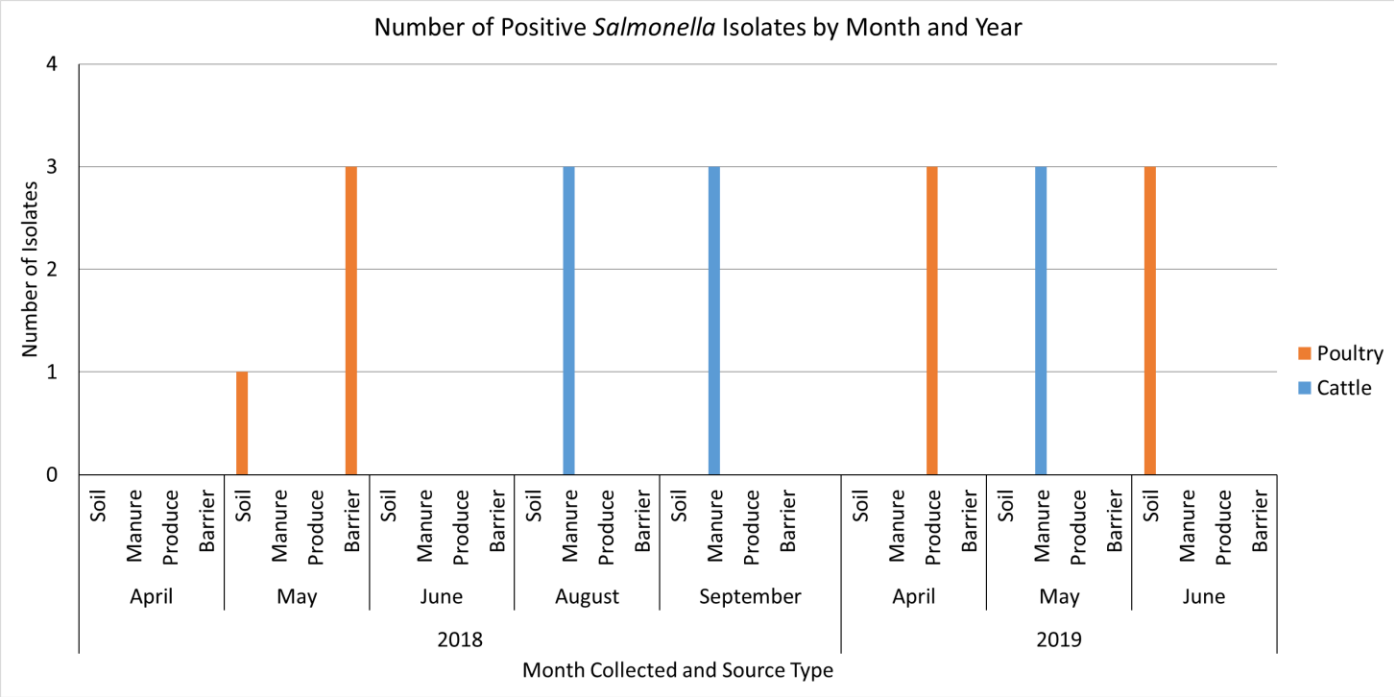

Figure 6 – 2018–2019 Prevalence of *Salmonella* isolates by month. *Salmonella* isolates were recovered from environmental samples collected in April 2019 (n=3), May 2018 and 2019 (n=7), June 2019 (n=3), August 2018 (n=3), and September 2018 (n=3).

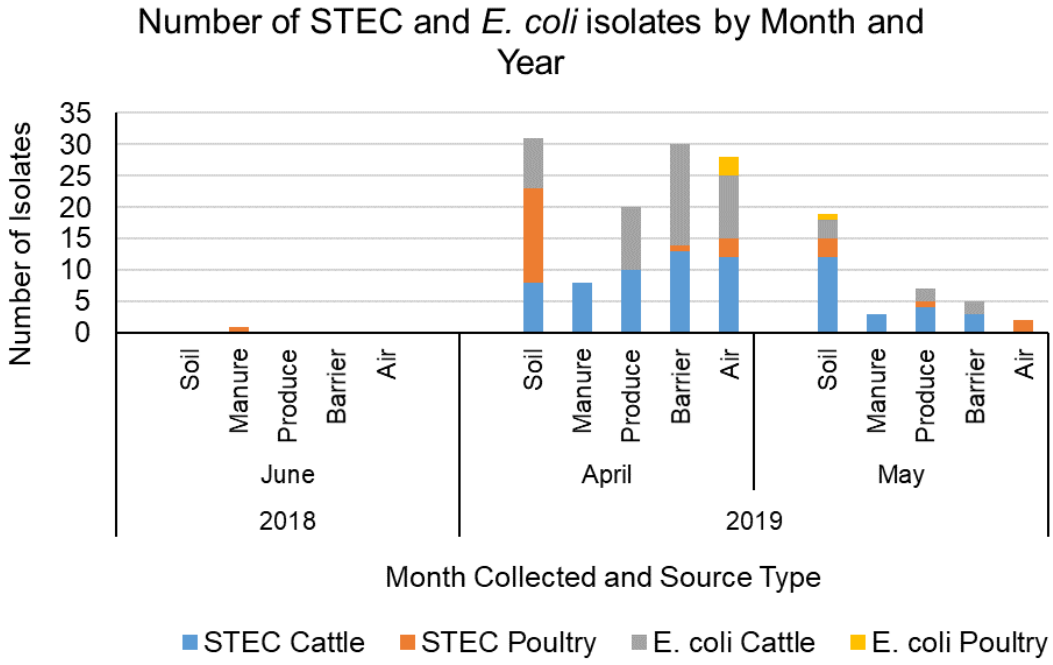

Figure 7 – 2018–2019 Prevalence of STEC and *E. coli* isolates by month. STEC isolates were recovered from environmental samples collected in June 2018 (n=1), April 2019 (n=70), and May 2019 (n=28). Generic *E. coli* isolates were recovered from samples in April 2019 (n=47) and May 2019 (n=8).

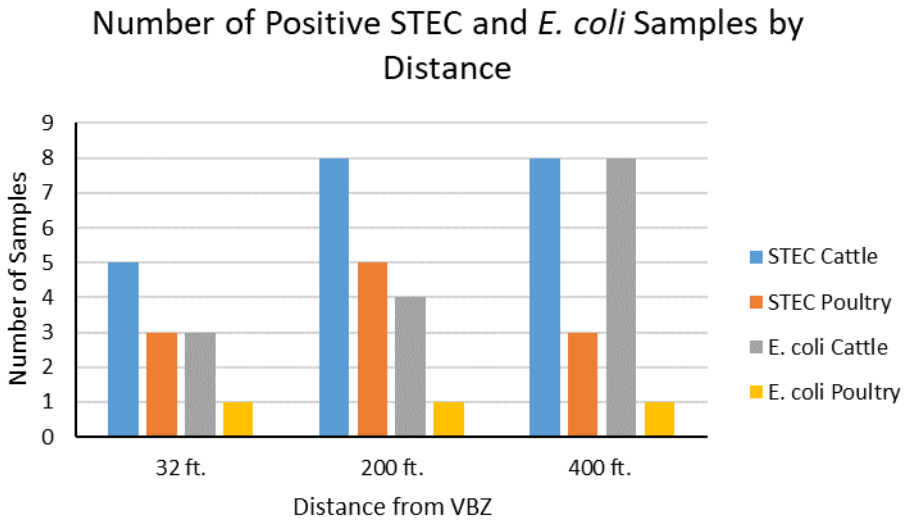

Figure 8 – Prevalence of positive STEC and *E. coli* samples by distance. STEC was recovered from farm samples collected at 32 ft (cattle: n=5; poultry: n=3), 200 ft (cattle: n=8; poultry: n=5), and 400 ft (cattle: n=8; poultry: n=3) from the VBZ. Generic *E. coli* was recovered from farm samples collected at 32 ft (cattle: n=3; poultry: n=1), 200 ft (cattle: n=4; poultry: n=1), and 400 ft (cattle: n=8; poultry: n=1) from the VBZ.

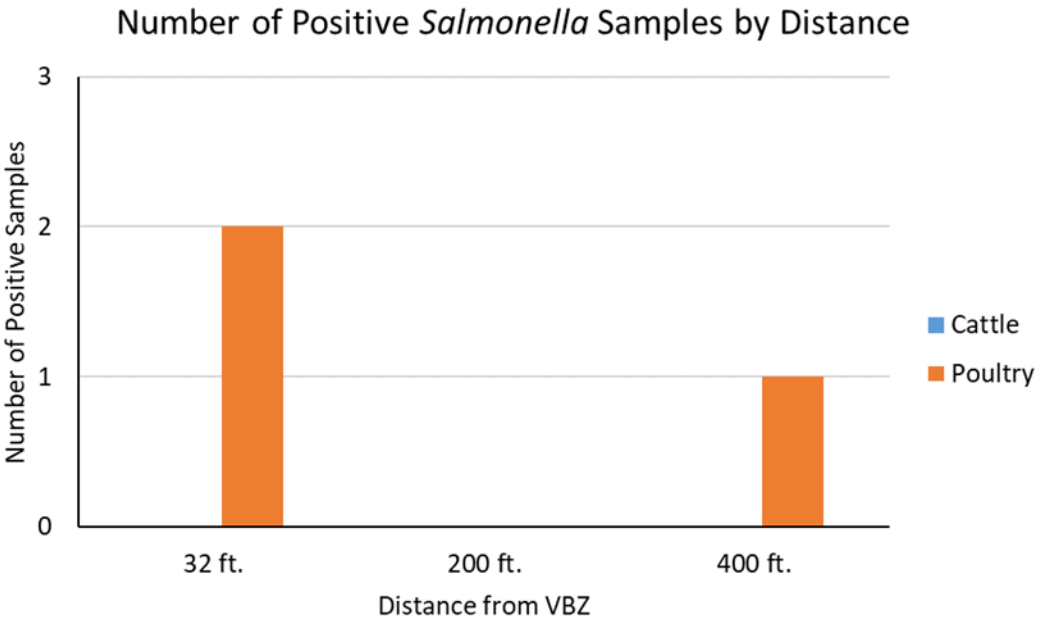

Figure 9 – Prevalence of *Salmonella*-positive samples by distance. *Salmonella* was recovered from two samples (soil and produce) collected at the poultry farm at 32 ft, and one soil sample collected at 400 ft from the poultry farm VBZ.

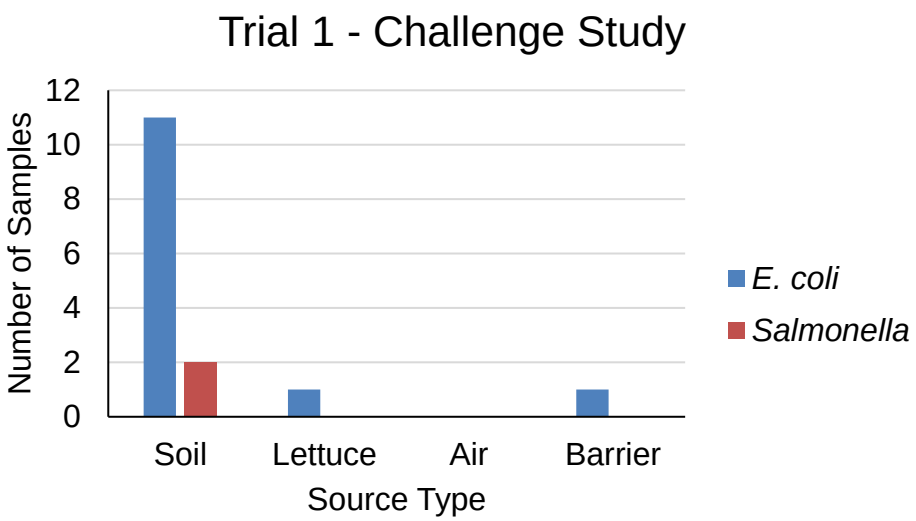

Figure 10 – Number of positive environmental samples in challenge trial 1. Rifampicin-resistant *E. coli* was recovered from dairy cattle farm soil (n=11), lettuce (n=1), and barrier plant (n=1) samples after the VBZ inoculation. Rifampicin-resistant *Salmonella enterica* Typhimurium was recovered from poultry farm soil samples (n=2) before inoculation. No air samples collected during the first trial were positive for *E. coli* or *Salmonella* before or after inoculation.

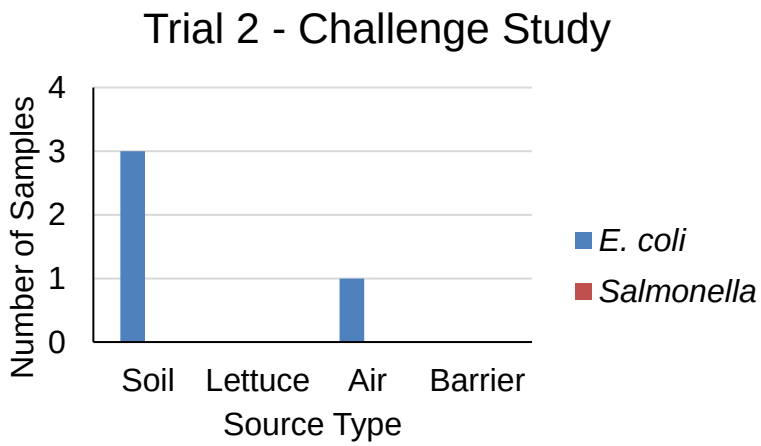

Figure 11 – Number of positive environmental samples in challenge trial 2. Rifampicin-resistant *E. coli* was recovered only from dairy cattle farm samples: from one air sample collected before inoculation and from soil samples (n=3) collected after VBZ inoculation. No produce or barrier plant samples collected during the second trial were positive for *E. coli* or *Salmonella* before or after inoculation.