CPS CENTER for PRODUCE SAFETY

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FINAL PROJECT REPORT

Project Title
Does *Salmonella* move through the irrigation systems of mixed produce farms of the southeastern United States?

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Objectives
The overall goal of our project team is to develop the knowledge which will allow vegetable producers that rely on untreated surface sources of irrigation water to effectively address new rules the FDA may implement on safe agricultural water. With that in mind, our proposal's direct goal is to understand if Salmonella moves through the irrigation systems of mixed produce farms of the southeastern United States and if so, if it persists on the crop until harvest and can be mitigated by treating the irrigation water with chlorine dioxide. To address this goal, we have developed the following objectives:

1. Sample irrigation systems on 5 farms to determine if the pilot project findings are representative of the overall condition and to determine which combinations of water source and irrigation system type are more prone to pathogen transport.

2. Determine if Salmonella strains in irrigation water contaminate produce and if the Salmonella persists on the crop until harvest.

3. Determine if chlorine dioxide treatment of irrigation water drawn from ponds effectively removes pathogens and prevents contamination of produce following irrigation.

4. Assess the validity of measuring generic E. coli as an indicator for Salmonella serovars under southeastern Coastal Plain conditions.

Funding for this project provided by the Center for Produce Safety through:
CPS Campaign for Research

FINAL REPORT

Abstract

The overall goal of this project was to develop the knowledge that will allow vegetable producers who rely on untreated surface sources of irrigation water to effectively address recently released rules on safe *agricultural water* included in the FDA's "Food Safety Modernization Act Final Rule on Produce Safety." FDA's definition of *agricultural water* is that which comes into *direct contact* with produce, and includes irrigation water that is applied using direct water application methods such as overhead sprinkler irrigation. The project's objectives were to understand if *Salmonella* moves through the irrigation systems of mixed produce farms of the southeastern United States; to determine if *Salmonella* is transferred to crops through irrigation water and if it persists on the crop until harvest in this environment; and to assess if pathogen loads in irrigation water can be effectively mitigated by disinfecting the irrigation water with chlorination. Because of our extensive database, which exceeds 500 water samples collected from surface irrigation sources and irrigation systems, we also assessed the validity of measuring generic *E. coli* as an indicator of *Salmonella*.

The project was conducted on four farms in southern Georgia. Southern Georgia is an ideal place to conduct the study because it is representative of much of the southeastern Coastal Plain, both in topography and climate but also in cultural practices used by vegetable producers. The project spanned two years (2014 and 2015). In Year 1, we analyzed 94 water samples from three pivot and two solid set sprinkler systems and three drip irrigation systems, and their water sources. The water sources were three irrigation ponds and one groundwater well. The analyzed samples were composites of several individual samples collected at each sampling event. Twenty-four of the 94 water samples (25%) were positive for *Salmonella*. Concentrations were consistently low, with the highest measured concentration being 0.99 MPN/100 mL—this sample was from one of the pivots. Five of six (83%) composite pivot samples were positive, which is considerably higher than measured in a pilot study (30%) and also higher than the ponds from which water was withdrawn (33%). Nine different serovars were identified in the water samples, with *S*. Muenchen and *S*. Saintpaul making up 65% of the isolates. *Salmonella* was detected on two produce samples: cantaloupe (pivot irrigation) harvested in late June and cucumber (drip irrigation) harvested in July. *Salmonella* serotypes were a *S. sp*. Rough "O" on the cantaloupe sample and *S. sp*. Rough "O" and *S.* Bardo on the cucumber sample. These serovars were not found in any of the irrigation water samples so other environmental factors, such as soil splashed by rain, may be responsible. Generic *E. coli* in water sample concentrations from the ponds and other irrigation systems were consistently below 50 MPN/100 mL with the exception of two samples. Both samples were collected on the same date from a pond (727 MPN/100 mL) and from the pivot withdrawing from the pond (649 MPN/100 mL). Both water samples were also positive for *Salmonella* and the pivot sample had the highest *Salmonella* concentration measured in the study. Overall, there was poor correlation between generic *E. coli* and *Salmonella* concentrations.

After reviewing product and scientific literature and discussions with the participating farms, we used a calcium hypochlorite tablet chlorination system to disinfect the irrigation water. Four Accu-Tab brand chlorinators were installed at the pumping stations of the irrigation systems withdrawing from ponds that were used in Year 1. Our grower partners requested that we maintain residual-free chlorine concentrations at no higher than 2 mg/L to avoid damage to the plants. During Year 2 we collected and analyzed 130 water samples. Chlorination did not eliminate the presence of *Salmonella* or generic *E. coli*, but there was a large reduction in the number of positive samples collected downstream of the chlorination injection point. The largest measured *Salmonella* concentration was 0.16 MPN/100 mL. Most of the positive *Salmonella* samples were at our detection limit of 0.055 MPN/100 mL. The largest measured generic *E. coli* concentration was 45.9 MPN/100 mL. An extension booklet on commercially available irrigation water disinfection methods was published.

Background

Irrigation is an essential component of fruit and vegetable production. Yet irrigation water has been shown to be a vector for the contamination of fresh produce by pathogenic bacteria and has been implicated in outbreaks of foodborne human disease and deaths (Greene et al., 2008). The U.S. Food and Drug Administration (FDA) recognizes this, and in its recently released "Food Safety Modernization Act Final Rule on Produce Safety," it establishes the requirement that all *agricultural water* must be *safe* and of adequate sanitary quality for its intended use (§ 112.41). FDA's definition of *agricultural water* is water that comes into *direct contact* with produce and includes irrigation water that is applied using direct water application methods such as overhead sprinkler irrigation. It does not include indirect water application methods such as drip or furrow irrigation. Section §112.42 requires producers to ensure that their water sources (including ponds) are safe, that the sources' watersheds are protected, and that their water distribution systems (such as irrigation systems) are free of pathogens. The Final Rule also requires regular sampling during the growing season of any untreated surface water (for example, a river or pond) that receives precipitation-driven surface runoff and is used as agricultural water. FDA also proposes that agricultural water be considered *safe* if test results do not exceeded a geometric mean of 126 CFU generic *E. coli* per 100 mL and a statistical threshold value of 410 CFU generic *E. coli* per 100 mL.

Although data may be available with which to respond to the FDA proposals on *safe agricultural water* for some parts of the United States, there are still significant knowledge gaps in the southeastern United States that prevent us from doing so. Before a science-based response can be formulated, this knowledge gap must be closed. Our project addressed this knowledge gap on *safe agricultural water* and specifically addressed irrigation water used by vegetable producers in the southeastern United States. The information from this region is also relevant to other agricultural regions of the United States that rely on untreated surface water and on-farm reservoirs as sources of irrigation water.

The southeastern Coastal Plain (SECP) is an ecoregion that spans portions of Louisiana, Mississippi, Tennessee, Alabama, Florida, Georgia, South Carolina, North Carolina, and Virginia (Figure 1). It is an important vegetable production area of the United States, with a long growing season that allows for at least two vegetable crops per year in most areas. Southern Georgia is in the heart of SECP and has been identified by federal agencies and researchers as being representative of the agricultural practices, climate, and water resources of the SECP (Jang et al., 2013; Cho et al., 2010; Sheridan et al. 1992).

In the SECP, a variety of irrigation sources are used by vegetable producers, with the most common source a constructed farm pond. These ponds are typically created by damming a 2nd or 3rd order stream. During the growing season, the ponds serve as source waters for on-farm irrigation systems. They are replenished by the stream, direct surface runoff during precipitation events, and sometimes by ground water from nearby wells. Even when a groundwater well is available, vegetable producers frequently irrigate directly from the pond because they can withdraw water from the pond at a much higher capacity than from the well. If the water is withdrawn for use in drip irrigation, it passes through a series of sand filters before entering the piping of the irrigation system. If the water is withdrawn for use in overhead sprinkler irrigation systems (center pivot or solid set), then it enters the piping of the irrigation system directly without any filtration.

Two companion CPS-funded studies, which were conducted in the SECP, have consistently found measurable concentrations of *Salmonella*, Shiga toxin-producing *E. coli* (STEC) (Gu et al., 2013a), and *Campylobacter jejuni* (Gu et al., 2013b) in water samples collected from ponds used for irrigation. Li et al. (2014) analyzed the same samples and found that nine *Salmonella* serovars were identified by pulsed-field gel electrophoresis analysis. The major serovar was *Salmonella enterica* serovar Newport (*S*. Newport, *n*=29, 57%), followed by *S. enterica* serovar Enteritidis (*n*=6, 12%), *S. enterica* serovar Muenchen (*n*=4, 8%), *S. enterica* serovar Javiana (*n*=3, 4%), *S. enterica* serovar Thompson (*n*=2, 4%), and other serovars. It is noteworthy that the PulseNet patterns of some of the isolates were identical to

those of the strains that were associated with the *S*. Thompson outbreaks in 2010, 2012, and 2013, the *S*. Enteritidis outbreaks in 2011 and 2013, and an *S*. Javiana outbreak in 2012.

During the 2012 spring/summer and the summer/autumn vegetable growing seasons in southern Georgia, Drs. Vellidis and Jay-Russell conducted a pilot study to assess the presence of *Salmonella* in irrigation water exiting four different irrigation systems used to irrigate tomatoes, squash, peppers, eggplant, cantaloupe, and two types of leafy greens on three farms. The irrigation systems included two drip systems, a center pivot system, and a solid set sprinkler system. One drip system was fed from a deep groundwater well and the other from a pond. Both the pivot and the solid set were fed from a single pond. Both ponds were included in other CPS-funded studies.

No *Salmonella* was detected in any samples collected from the well or from the very extensive drip system fed from the well (Table 1). In contrast, *Salmonella* was found in samples collected from all three irrigation systems fed by pond water. Approximately 30% of the samples collected from the pivot were positive for *Salmonella* (Table 1). Likewise, *Salmonella* was found in approximately 37% of the samples collected from the end of the drip lines in the drip system fed from the pond. The number of positive samples was higher in the summer than either in the early spring or late autumn. The solid set sprinkler system was used to irrigate leafy greens during late autumn, and we collected samples from it only during November and December. Only two positive samples were collected during this period: one from Pond 1, which served as the water source, and one directly from the irrigation system. Generic *E. coli* concentrations in all samples from the irrigation systems containing *Salmonella* were below the geometric mean of 126 CFU/100 mL (Table 1). Generic *E. coli* were found only in samples associated with pond water and irrigation systems fed by pond water. No samples associated with the well or the drip system fed by the well tested positive for generic *E. coli.* These preliminary findings were quite telling about the differences between water sources and irrigation systems, but the sample sizes were small and this led us to conduct the study reported here in order to draw defensible conclusions. This study provides vegetable producers in the Southeast and by extension, nationwide, with knowlege to effectively address FDA rules on *safe agricultural water*.

Research Methods and Results

The project was conducted on four farms in southern Georgia, all of which were located within the Little River watershed (Figure 1). Southern Georgia is an ideal place to conduct the study because, as explained earlier in the report, this area is considered representative of much of the southeastern Coastal Plain, both in topography and climate but also in cultural practices used by vegetable producers. Three of the four farms were sampled in previously funded CPS studies. Having longitudinal data over time is a significant strength of this study. Three of the four farms use ponds to supply irrigation water to either overhead or drip irrigation systems (or both). The fourth farm uses well water to irrigate through a drip system. During the life of the project, we collected samples from irrigation systems watering bell pepper, cantaloupe, collard greens, cucumbers, mustard greens, okra, squash, tomatoes, watermelon, and zucchini.

Objective 1 – Sampling Irrigation Systems on 5 Farms

From 05 May to 05 November 2014 (Year 1 of the project) we analyzed 94 water samples from three pivot sprinkler, two solid set sprinkler, and three drip irrigation systems. The analyzed samples were composites of several individual samples collected at each sampling event. Specifically, for drip systems, samples were collected at the beginning of three randomly selected drip lines and then composited, and samples were collected from the end of the same three drip lines and then composited. In the field, samples were collected directly into sterile 1-L plastic sample bottles, placed in a cooler filled with ice and transported to the UGA Water Quality Laboratory (UGA WQL) where they were composited. For center pivot irrigation systems, six samples were collected from randomly selected sprinklers along the

length of the pivot and composited. For solid set sprinkler systems, six samples were collected from randomly selected sprinklers throughout the field in sterile 1.5-L catch cups and then immediately poured into sterile 1-L plastic sample bottles. The bottles were returned to the laboratory where they were composited. Figure 2 shows how samples were collected from the different irrigations systems.

At each irrigation event, samples were also collected from the irrigation water source. If the source was a pond, a sample was collected from the pond near the intake of the irrigation system pumping system by using a peristaltic pump equipped with sterile Tygon tubing (Figure 3). Samples were collected from a depth of approximately 1 m while the irrigation pump was operating. Samples were also collected from a water valve/spigot installed immediately downstream of the irrigation system's pump and prior to any filters (Figure 2). In addition, a multiparameter water quality sonde was used to measure *in situ* temperature, pH, dissolved oxygen concentration, turbidity, and specific conductivity at each pond (Figure 3). If the irrigation water source was the well, the source water sample was collected from the water valve/spigot as described earlier.

Finally, precipitation events at each pond were recorded with a tipping bucket rain gauge connected to a data logger. The rain gauges were installed on the bank nearest the intake. Data were downloaded monthly during the regular sampling visits to the ponds.

Water Sample Analyses

All samples were analyzed for *Salmonella*, generic *E. coli*, total coliforms, NO_3-N, NH_4-N, TN, PO_4-P, TP, Cl^-, and total suspended solids under the supervision of Dr. Moukaram Tertuliano and Ms. Debbie Coker at the UGA WQL. A novel cross-streaking method developed by Dr. Anita Wright (Luo et al., 2014) was used to isolate, confirm, and enumerate *Salmonella*. The method uses a MPN protocol with three dilutions cultured in enrichment broth, selective broth, and plated on two types of selective media. With the dilutions we were using, our lower detection limit was 0.0548 MPN/100 mL and our upper detection limit was 11 MPN/100 mL. All presumptive positive *Salmonella* colonies were confirmed by PCR targeting the *InvA* gene in Dr. Sree Rajeev's laboratory at the University of Georgia's Veterinary Diagnostic Laboratory in Tifton. All isolates confirmed positive by PCR were frozen and shipped quarterly to Dr. Jay-Russell at the Western Center for Food Safety at the University of California, Davis, for molecular typing by pulsed-field gel electrophoresis (PFGE) using the CDC PulseNet protocol (Ribot et al., 2006). (PFGE results are described separately below.)

The IDEXX Colilert reagent and the Quanti-tray system were used to analyze water samples for generic *E. coli* (IDEXX Laboratories, 2013). Samples were analyzed for NO_3-N, NH_4-N, TN, PO_4-P, TP, Cl^- with colorimetric autoanalyzers using standard methods (Clesceri et al., 1998).

Salmonella and Generic *E. coli* Results

Twenty-four of the 94 water samples (25%) were positive for *Salmonella*. Concentrations were consistently low, with the highest measured concentration being 0.99 MPN/100 mL. The one sample with this concentration was from one of the pivots. Table 2 presents a summary of the samples collected and analyzed during the study. In Table 2, the Year 1 – 2014 data are associated with Objective 1. Year 2 – 2015 data pertain to Objective 3 and will be discussed later. The results from well spigot, well drip irrigation systems, the ponds, the pond spigots and the pond drip systems are all consistent with the results from our pilot study. The pond results are also consistent with other CPS-funded studies that examined the biological water quality of two of the three ponds used in the current study.

The six pivot samples (each sample was a composite of six individual samples) were collected from two different pivots on different farms (three sampling events per pivot). Both pivots withdraw water from a pond. Only the first sample collected on 06 May 2014 was negative. The other five samples collected between 20 May and 30 June were positive, resulting in an 83% positive rate, which is considerably higher than we measured in our pilot study (30%) and also higher than the ponds from

which water was withdrawn. One of the two ponds was positive on two of the sampling events associated with the pivot sampling. The other pond was not positive on any of the three pivot sampling events, but the pivot using the pond was positive on all three events. The same pivot was also used in our pilot study, and at that time we swabbed the biofilm within the mainline of the pivot and analyzed the swabs for *Salmonella*; there were no positives from the biofilm swabs. Table 3 presents the results of the statistical analyses of the *Salmonella* data. Only the *Salmonella* samples collected directly from center pivot irrigation systems had concentrations that were statistically significantly different than other samples.

As with the related pilot irrigation water study we conducted in 2012, no generic *E. coli* were found in any samples collected from the groundwater well used or from the drip irrigation system fed by the well. Sample concentrations from the ponds and other irrigation systems were consistently below 50 MPN/100 mL, with the exception of two samples. Both samples were collected on 18 June 2014: one from the pond (727 MPN/100 mL) and one from the pivot withdrawing from the pond (649 MPN/100 mL). Both water samples were also positive for *Salmonella*, and the pivot sample had the highest *Salmonella* concentration measured in the study. Table 4 presents the results of statistical analyses for the generic *E. coli* data. The mean of the pivot samples was more than twice as large as the mean of the pond samples. However, there were no statistically significant differences between these two sample groups because of the large variability between the data. There were statistically significant differences between the pivot samples and pond spigot, drip tape start (pond), drip tape start and end (well), and the well spigot.

Nutrients and Suspended Solids

All NO_3-N, NH_4-N, PO_4-P, Cl^- and suspended solids analyses have been completed. All samples have been digested for TN (total N) and TP (total P) analysis but only the 94 samples collected during 2014 have been analyzed to date. Analysis of the 2015 samples for TN and TP was delayed by an instrument failure but analysis is currently in progress. The nutrient results will be submitted to CPS as an addendum to this report when they do become available.

PFGE Results

From the samples collected in 2014, confirmed *Salmonella* isolates cultured from water (103) and produce (5) rinsates were shipped by the University of Georgia (water) and Emory University (produce) to Dr. Jay-Russell at the Western Center for Food Safety (WCFS) to be further analyzed by PFGE. Positive *Salmonella* controls that were used throughout the experiment were collected environmentally from regions in Georgia or Florida and were also analyzed with PFGE. Once at the WCFS, isolates were cultured, confirmed by PCR, and stored at -80°C in micro banks (Pro-Lab, Richmond Hill, OH). PFGE was performed on all isolates according to a protocol developed by the Centers for Disease Control and Prevention (CDC) (Ribot et al., 2006) using *Salmonella enterica* serotype Braenderup H9812 as a control strain. Briefly, agrose-embedded DNA was digested with 10U of *XbaI* (Roche, San Francisco, CA) for 3 hours in a water bath at 37°C. The restriction fragments were separated by electrophoresis in 0.5X Tris-borate-EDTA (TBE) buffer (Corning, Manassas, VA) at 14°C for 19.5 hours using a CHEF Mapper electrophoresis system (Bio-Rad, Hercules, CA). Isolates showing DNA smears were retested by digesting plugs with *XbaI* and adding 50µM thiourea (Sigma-Aldrich, St. Louis, MO) to the running buffer of 0.5X TBE. The gels were stained with ethidium bromide, and DNA bands were visualized with UV transillumination (Bio-Rad). PFGE results were analyzed using BioNumerics software 7.1 (Applied Maths, Kortrijk, Belgium). A dendrogram was constructed using band-band analysis with an optimization of 2.5%, band-matching tolerance at 1.5%, followed by the UPGMA method for clustering.

Twenty-six unique pulsotypes were determined by PFGE analysis. There were 6 pulsotype matches when comparing the pulsotypes found in irrigation water sources and those found in irrigation water.

To better visualize these pulsotype data and MPN levels at each irrigation water source, plots for each irrigation source were created (Figure 4). For each plot, samples were stratified by sample type (source water, irrigation distribution system, produce). Samples that were negative were plotted in black. Positives samples were plotted in varying colors by pulsotype. Unique pulsotypes that were only associated with one isolate were plotted in grey and labeled "Singleton."

As indicated by the dendrogram (Figure 5) and pulsotype plots, a variety of strains were detected in these produce and water samples. While no strains were detected throughout the entire chain from water source (ponds) to irrigation distribution systems to produce, there were some matches between ponds and irrigation systems. Some strains were very common and were present at multiple ponds and irrigation systems while others were limited to specific sites. Additionally, there was within-sample diversity of strains—one sample had 10 unique pulsotypes detected. The diversity in these irrigation systems was also dynamic. Many pulsotypes detected in the pilot study from 2012–2013 were not detected in samples from the same irrigation system in 2014 and pulsotypes not previously detected emerged in the 2014 samples.

Serotyping

Of the 108 isolates from produce and water samples, 8 isolates (3 produce rinsate samples, 5 water samples) were sent to the California Animal Health and Food Safety Lab, University of California, Davis, to be serotyped. An additional 36 isolates were sent for serotyping to the National Veterinary Services Laboratory (NVSL) in Ames, Iowa. Some sample results are still pending. Table 5 presents the water sample serotyping results which are currently available and shows prevalence of serovars across all water samples. Nine different serovars were identified in the water samples with *S*. Muenchen and *S*. Saintpaul making up 70% of the isolates. The majority of serovors found in water samples of this study were also found in the water samples of the 10 pond survey conducted in 2011–2012 (Li et al., 2014) and the irrigation water pilot study conducted in 2012 (Antaki et al., 2016). There were some instances when the same serovar was found in the source water and the irrigation system on the same sampling date. For example, *S*. Muenchen and *S*. Saintpaul were found in the pond water sample and the pivot sprinkler water sample on 18 June 2014.

Objective 2 – Determine if Irrigation Water Contaminates Produce

During 2014 (Year 1) we collected and analyzed 65 produce samples from bell pepper, broccoli, cantaloupe, collard greens, cucumbers, mustard greens, squash, watermelon, and zucchini (Table 6). All crops were harvested from fields in which we collected irrigation water samples, but the broccoli and collard greens samples were harvested before we began collecting water samples from the irrigation system. Prior to the commercial harvest of each crop, we harvested five produce samples in sterile Whirl-Pak bags from different locations in the field. The units of sample per bag varied by crop as indicated in Table 6. For example, we collected two cantaloupes or six cucumbers or six collard green leaves per bag. The samples were stored on ice until they arrive at the laboratory where they were analyzed individually. At the laboratory, 2 L of peptone were added to each of the six bags to wash the produce (30 s shaking, 60 s massaging, 30 s shaking). The rinsate was then poured into replicate bottles containing lactose broth. There were 9 replicate bottles per sample: 3 bottles with 500 mL lactose and 500 mL rinsate, 3 bottles with 100 mL lactose and 100 mL rinsate, and 3 bottles with 10 mL lactose and 10 mL rinsate. The replicate bottles were incubated for 24 hours at 37°C. After 24 hours, 8.5 mL of each incubated bottle was mixed with 1.5 mL of glycerol and then frozen at -80°C for 90 days. Because we were working in commercial fields, pathogen testing of produce samples was delayed for 90 days. After 90 days, samples were thawed to room temperature for 2 hours and incubated at 37°C for 2 hours. Then, 1 mL of sample was added to 10 mL of tetrathionate broth. The sample was incubated at 37°C for 24 hours and then streaked onto XLT4 agar plates.

Salmonella was detected on two produce samples: cantaloupe harvested in late June and cucumber harvested in July (Table 6). The cantaloupe was grown on narrow bed plastic and irrigated by pivot using water from a pond. The cantaloupe sample was collected on 20 June, which was 2 days after water samples from the pond and the pivot were positive for *Salmonella*. Those two water samples had our two highest measured *Salmonella* and generic *E. coli* concentrations. Serotyping determined that the *Salmonella* isolate from the cantaloupe sample was *S. sp*. Rough "O", while the isolates from the pivot water samples were either *S.* Muenchen, *S.* Saintpaul, or *S*. Javiana, and the isolates from the pond water sample were either *S.* Muenchen, *S*. Saintpaul, or *S*. Rubislaw.

The cucumber was grown on bed plasticulture with drip tape under the plastic, using water from a pond. PFGE analysis indicates that the pulsotype found on the cucumber sample did not match the pulsotypes found in the irrigation water and pond samples. The two *Salmonella* isolates from the cucumber sample were serotyped as *S. sp*. Rough "O" and *S.* Bardo. Serotyping results for water samples collected prior to the produce harvest are not yet available, but other water samples from that pond and irrigation system are not associated with the serovars found on the cucumber. Other environmental factors such as soil splash from rain may be responsible for contaminating the two produce samples.

Objective 3 – Does Chlorination of Irrigation Water Effectively Remove Pathogens

After an extended review of product and scientific literature and discussions with the participating farms, we used a calcium hypochlorite tablet chlorination system to treat the irrigation water instead of injecting chlorine dioxide as originally proposed. We made this change for several reasons, but the primary reason was grower preference for an easier-to-use chlorination process. The literature shows that calcium hypochlorite tablet chlorination systems perform as effectively as chlorine dioxide injection systems. Furthermore, one of the participating farms had experience with this type of system and their data showed that residual free chlorine concentrations of 2 to 4 mg/L could be achieved in irrigation systems fed by pond water. Four Accu-Tab brand chlorinators were purchased and were installed during the first week of April 2015 (Year 2 of the project) at the pumping stations of the irrigation systems withdrawing from ponds that were used in Year 1 of the study (Figure 6). The growers requested that we maintain residual free chlorine concentrations at no higher than 2 mg/L because of concerns that higher concentrations may damage the plants. We spent the month of April calibrating the chlorination systems to ensure that we achieved the desired residual free chlorine concentrations in the irrigation systems. Each of the systems required a different number of calcium hypochlorite tablets to account for the concentration of suspended solids in the water column (Figure 6).

We began collecting water samples from the chlorinated irrigation systems in early May. Samples were collected from three ponds, spigots at four pond pumping stations (there are two pumping stations at one pond), and the irrigation systems used to irrigate produce fields around the ponds. The four pumping stations fed three center pivot irrigation systems, one solid set irrigation system, and two drip irrigation systems. We also collected samples from all of these irrigation systems during 2014. We collected and analyzed 130 water samples between 04 May and 01 December 2015. Samples were analyzed for *Salmonella*, generic *E. coli*, total coliforms, total suspended solids, NO_3-N, NH_4-N, total N, PO_4-P, total P, and Cl^-. Table 2 presents and compares bacterial analyses results for samples collected in 2014 (without chlorination) and samples collected in 2015 (with chlorination). The percent of samples positive for *Salmonella* and generic *E. coli* remained about the same (around 32%) in the pond water for both years. Although chlorination did not eliminate the presence of *Salmonella* or generic *E. coli* from the water in the irrigation systems, there was a marked reduction in the number of positive samples collected downstream of the chlorination injection point. Even the spigot/valves which are immediately downstream of the injection point showed a 50% reduction in the percent positives.

The largest measured *Salmonella* concentration was 0.16 MPN/100 mL from a drip tape sample collected on 26 May 2015. Most of the positive *Salmonella* samples were at our detection limit of 0.055 MPN/100 mL. The largest measured generic *E. coli* concentration was 45.9 MPN/100 mL from a spigot/valve sample. Tables 7 and 8 present a statistical comparison of mean *Salmonella* and generic *E. coli* concentrations of the water samples during chlorination. There were no statistically significant differences between the sample groups.

2015 Produce Results

During 2015 we collected and analyzed 30 produce samples from cantaloupe, collard greens, cucumber, and tomato (Table 6). The samples were collected and analyzed using the methods described under Objective 2. None of the 20 samples analyzed to date were positive for *Salmonella* but results from 10 samples that are still frozen are pending. The results will be submitted to CPS as an addendum to this report when they do become available.

Extension Booklet on Irrigation Water Disinfection Methods

Dr. Elizabeth Antaki, a Post-Doctoral Research Associate working under Dr. Jay-Russell's supervision at UC Davis, conducted a review of commercially available methods for disinfecting irrigation water. She integrated this information into a 4-page extension booklet that describes the methods and compares their advantages and disadvantages. This booklet will provide fruit and vegetable growers with valuable and non-biased information about irrigation water disinfection systems. Figure 7 presents the cover of the booklet and a summary table of the disinfection methods found in the booklet (p. 3). One hundred copies will be printed for distribution but it will also be made widely available through online resources at UC Davis and the University of Georgia.

Objective 4 – Assess the validity of measuring generic *E. coli* as an indicator for *Salmonella*

Between 2012 and 2013, we collected in excess of 500 water samples, all of which we analyzed for *Salmonella* and generic *E. coli* concentrations. This provides us with an unparalleled data set with which to conduct a rigorous statistical analysis to assess validity of measuring generic *E. coli* as an indicator for *Salmonella*. Table 9 compares FDA FSMA agricultural water quality generic *E. coli* thresholds to occurrence of *Salmonella* in water samples collected during a CPS-funded project from 2012–2013. Figure 8 shows that there is also poor correlation between generic *E. coli* and *Salmonella* concentrations in this landscape. The statistical analysis of the comprehensive data set is currently in progress and the results will be submitted to CPS as an addendum to this report when they do become available.

Outcomes and Accomplishments

This project provided many challenges ranging from logistical to scientific but resulted in a series of positive outcomes and accomplishments. With support from the Center for Produce Safety, we developed a strong and dynamic multi-state, multi-institutional team dedicated to developing knowledge that will allow vegetable producers who rely on untreated surface sources of irrigation water to effectively address recently released FDA rules. The partner institutions include the University of Georgia, Emory University, the University of California at Davis, and the Western Center for Food Safety. The team consisted of microbiologists, water quality experts, hydrologists, and several vegetable producers in southern Georgia. The partnerships and trust we developed with the vegetable producers will be long-lasting and will allow us to conduct important on-farm projects in the future. In addition, we trained several young scientists during the project's two years. One Ph.D. student conducted a component of her Ph.D. dissertation work on this project. We also employed three post-doctoral researchers (one at each university), three undergraduate student workers, a field technician and a lab analyst.

We continued to use and improve innovative analytical techniques for the laboratory, which we developed during previous CPS-funded projects and adopted innovative techniques for analyzing produce collected from commercial fields. We developed innovative sampling techniques for the field, which will make future projects easier, more cost-effective, and more productive.

We were excellent stewards of the Center for Produce Safety funds provided to us for this project. The existing infrastructure and technical expertise at Dr. Sree Rajeev's laboratory at the University of Georgia's Veterinary Diagnostic Laboratory in Tifton allowed us to expeditiously confirm all presumptive positive *Salmonella* colonies by PCR targeting the *InvA* gene at a relatively low cost despite the fact that we analyzed a much larger number of samples by PCR than originally expected. We were also able to leverage the project's personnel costs with related ongoing projects and thus realized significant savings, and we were able to analyze a significantly larger number of water samples during 2015 than committed to in the original proposal.

We successfully completed the goals of our proposal although the experimental approach used for our second objective (chlorination) was slightly modified from that originally proposed to conform to grower preferences. The results from this study provide vegetable producers in the Southeast and by extension, nationwide, with knowlege to effectively address new FDA rules on *safe agricultural water*. Our findings and recommendations are described in the following section.

Summary of Findings and Recommendations

- Approximately 32% of the water samples collected from the irrigation ponds used in this study were positive for *Salmonella*. Concentrations were low and consistently below 1 MPN/100 mL. Generic *E. coli* were not found in all pond samples and all pond concentrations were below 70 MPN/100 mL, with the exception of one sample. These data are consistent with findings from previous studies conducted by our team in the same ecoregion (Antaki et al., 2016; Li et al., 2014, 2015; Luo et al., 2014, 2015; Gu et al., 2013a, 2013b).
- The bacteria pass through the irrigation systems at about the same rates as found in the ponds regardless of whether irrigation is by overhead sprinkler or drip systems.
- Of 65 produce samples from untreated systems, two were found positive for *Salmonella*: one cucumber sample and one cantaloupe sample. PFGE and serotyping did not detect any matches between *Salmonella* isolated from produce and *Salmonella* isolated from irrigation water. Other environmental factors may be responsible for contaminating the two produce samples. One potential contamination pathway may be soil splashed on the produce by rain.
- Irrigation water disinfection, in this case a calcium hypochlorite tablet chlorination system calibrated for a <2 mg/L free chlorine residual, resulted in greater than 50% reductions in the percentage of samples positive for *Salmonella* and an even greater reduction in the percentage of samples positive for generic *E. coli.* No produce samples were found positive for *Salmonella* when irrigation water was chlorinated.
- A 4-page extension booklet titled "Pre-harvest irrigation water: Methods for disinfection" was developed and will provide fruit and vegetable growers with valuable and non-biased information about irrigation water disinfection systems.
- FSMA guidelines based on generic *E. coli* are not a good indicator for the presence of *Salmonella* in the ponds and irrigation systems of the southeastern Coastal Plain, as they result in an approximately 42% false negative rate.

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APPENDICES

Publications and Presentations

Abstracts

Lee, D., G. Vellidis, M. Tertuliano, E. Antaki, C. Harris, M. Jay-Russell, and K. Levy. 2016. *Salmonella* transport through irrigation systems and the risk of fresh produce contamination on farms in Southern Georgia. 2016 International Association for Food Protection Annual Meeting, St. Louis, 31 July – 03 August (scheduled). https://www.foodprotection.org/annualmeeting/

Tertuliano, M., G. Vellidis, C. Harris, S. Rajeev, K. Levy. 2015. *Salmonella* transport from pond water sources through irrigation systems on mixed produce farms in the southeastern United States. Water Microbiology Conference 2015 Abstract Book, p.46, University of North Carolina at Chapel Hill. https://waterinstitute.unc.edu/files/2015/05/Abstract-book-Final.pdf

Professional Presentations

Lee, D., G. Vellidis, M. Tertuliano, E. Antaki, C. Harris, M. Jay-Russell, and K. Levy. 2016. *Salmonella* transport through irrigation systems and the risk of fresh produce contamination on farms in Southern Georgia. 2016 International Association for Food Protection Annual Meeting, St. Louis, 31 July – 03 August (scheduled).

Vellidis, G. and K. Levy. 2016. Is Your Irrigation Water Safe? Eastern Cantaloupe Growers Association Annual Meeting, Atlanta, Ga, 18 February.

Vellidis, G. and K. Levy. 2016. Is Your Irrigation Water Safe? Southeast Regional Fruit and Vegetable Conference, Savannah, Georgia, 08 January.

Levy, K. 2015. *Salmonella* in an Agricultural Landscape- Case Study in a Fresh Produce Growing Region of Southern Georgia. Centers for Disease Control NCEZID/DFEWD Division Seminar, Atlanta, GA, 11 December.

Vellidis, G. and K. Levy. 2015. Bringing Food Safety Research into Focus: A Case Study. 2015 Center for Produce Safety Research Symposium, Atlanta, Georgia, 23 June.

Vellidis, G. 2015. Is *Salmonella* Present in the Irrigation Water of Mixed Produce Farms in Georgia? Southeast Regional Fruit and Vegetable Conference, Savannah, Georgia, 06 January.

Conference Posters

Harris, C., D. Lee, E. Antaki, K. Levy, M. Jay-Russell, and G. Vellidis. 2015. Salmonella transport through irrigation systems and occurrence on crops at harvest. Southeast Regional Fruit and Vegetable Conference, Savannah, Georgia, 06 January.

Tertuliano, M., G. Vellidis, C. Harris, S. Rajeev, E. Antaki, M. Jay-Russell, D. Lee, and K. Levy. 2015. Does *Salmonella* move from water sources through irrigation systems on mixed produce farms? A case study in the Southeastern US. Water Microbiology Conference 2015, University of North Carolina at Chapel Hill.

Media

Center for Produce Safety – A Closer Look. 2015. Projects Follow *Salmonella's* Route through Irrigation Water.
http://www.centerforproducesafety.org/article/113/CPS_A_Closer_Look_Projects_Follow_Salmonellas_Route_Through_Irrigation_Water.html

Budget Summary

This summary reflects funds allocated to the University of Georgia. Supplies and Materials line item includes a subcontract to Emory University. The table does not include funds allocated directly to UC Davis.

Salaries Paid	$ 136,340.27
Benefits	$ 54,819.59
Travel	$ 712.33
Supplies and Materials	$ 105,070.01
Indirect Costs	$ 7,136.80
Total	**$ 304,079.00**

Tables and Figures

Table 1. Results from the 2012 pilot study to survey pathogens in irrigation system water.

Irrigation Water Source	No. of Samples	*Salmonella*		Generic *E. coli* (CFU/100 mL)		
		No. Positive	% Positive	GM	Min	Max
Pond 1 and Pond 2						
Surface water	25	5	20.00%	5.7	0	40
Subsurface water (1 m)	25	5	20.00%	4.4[1]	0	TNTC[2]
Pivot sprinkler heads	27	8	29.63%	5.7	0	18.9
Solid set sprinkler heads	24	1	4.17%	2.5	0	8.4
Start of drip line	24	5	20.83%	4.3	0	13.7
End of drip line	24	9	37.50%	2.9	0	6.3
Well 1						
From well pump	12	0	0.00%	0	0	0
Start of drip line	20	0	0.00%	0	0	0
End of drip line	20	0	0.00%	0	0	0
Total	**219**	**34**	**15.53%**			

[1] Does not include one value recorded as TNTC (too numerous to count).
[2] Second highest value was 12.6 CFU/100 mL

Table 2. Results from the 2014-2015 study to survey pathogens in irrigation system water.

Sample Source	*Salmonella*				Generic *E. coli*			
	Total	Negative	Positive	% Positive	Total	Negative	Positive	% Positive
Year 1 – 2014 (without Chlorination)								
Spigot (Well)	5	5	0	0.0	5	5	0	0.0
Drip (Well)	11	11	0	0.0	11	11	0	0.0
Pond	24	16	8	33.3	24	2	22	91.7
Spigot (Pond)	15	11	4	26.7	15	3	12	80.0
Pivot (Pond)	6	1	5	83.3	6	0	6	100.0
Solid Set (Pond)	3	3	0	0.0	3	2	1	33.3
Drip (Pond)	30	23	7	23.3	30	3	27	90.0
Total	94	70	24	25.5	94		94	100.0
Year 2 – 2015 (with Chlorination)								
Pond	35	24	11	31.4	35	8	27	77.1
Spigot (Pond)[1]	35	29	6	17.1	35	19	16	45.7
Pivot (Pond)	8	7	1	12.5	8	6	2	25.0
Solid Set (Pond)	2	2	0	0.0	2	1	1	50.0
Drip (Pond)	50	47	3	6.0	50	38	12	24.0
Total	130	109	--	16.2	130	109	21	--

[1] Rows shaded in BLUE are samples collected downstream of the chlorination system.

Table 3. Statistical comparison of mean *Salmonella* concentrations (MPN/100mL) of water samples collected during 2014; (pond) and (well) indicate the water source for the irrigation system (without Chlorination). Means with the same t Grouping letter are not significantly different. Data were analyzed using an analysis of variance GLM procedure follow by means separation LSD test.

t Grouping	Mean *Salmonella* (MPN/100mL)	N	Sample type
A	0.20633	6	Pivot (pond)
B	0.03583	24	Pond
B	0.02653	15	Spigot/valve (pond)
B	0.01920	15	Drip tape end (pond)
B	0.01593	15	Drip tape start (pond)
B	0.00000	6	Drip tape start (well)
B	0.00000	5	Drip tape end (well)
B	0.00000	3	Sprinkler
B	0.00000	5	Spigot/valve (well)

Table 4. Statistical comparison of mean generic *E. coli* concentrations (MPN/100mL) of water samples collected during 2014; (pond) and (well) indicate the water source for the irrigation system (without Chlorination). Means with the same t Grouping letter are not significantly different. Data were analyzed using an analysis of variance GLM procedure follow by means separation LSD test.

t Grouping		Mean *generic E. coli* (MPN/100mL)	N	Sample type
	A	112.92	6	Pivot (pond)
B	A	40.45	24	Pond
B	A	14.83	3	Sprinkler
B	A	13.33	15	Drip tape end (pond)
B		9.21	15	Spigot/valve (pond)
B		7.87	15	Drip tape start (pond)
B		0.00	6	Drip tape start (well)
B		0.00	5	Drip tape end (well)
B		0.00	5	Spigot/valve (well)

Table 5. Prevalence of *Salmonella enterica* in 37 water samples collected during 2014.

***S. enterica* Isolate**	**No. of PFGE Patterns**	**No. of Isolates**	**Percent of Total**
S. Saintpaul	10	15	40.5
S. Muenchen	6	9	24.3
S. Mbandaka	1	4	10.8
S. Hartford	1	2	5.4
S. Newport	2	1	2.7
S. Javiana	1	1	2.7
S. Rubislaw	1	1	2.7
III_16:z10:e,n,x,z15	1	1	2.7
III_60:r:e,n,x,z15	1	1	2.7
Pending[1]	2	2	5.4

[1] Results from NVSL still pending.

Table 6. Summary of produce samples collected during the project.

Year	**MM-DD**	**Irrigation System (source)**	**Produce**	**Units per Sample**	**Number of Positive Samples**	**MPN/Sample**	***Salmonella* Serovars**
Year 1 – 2014							
2014[1]	02-13	Pivot (pond)	Broccoli	5	0	<0.055	
2014	04-08	Solid set (pond)	Greens	6	0	<0.055	
2014	05-28	Drip (pond)	Squash	6	0	<0.055	
2014	06-20	Pivot (pond)	Cantaloupe	2	1	0.055	*S.* sp. Rough "O"
2014	06-20	Drip (well)	Bell Pepper	6	0	<0.055	
2014	06-23	Drip (pond)	Watermelon	1	0	<0.055	
2014	07-02	Pivot (pond)	Cantaloupe	2	0	<0.055	
2014	07-28	Drip (pond)	Cucumber	8	1	0.055	*S.* sp. Rough "O" *S.* Bardo
2014	08-31	Drip (well)	Cucumber	8	0	<0.055	
2014	10-06	Drip (pond)	Bell Pepper	6	0	<0.055	
2014	10-30	Drip (pond)	Zucchini	8	0	<0.055	
2014	11-07	Solid set (pond)	Greens	30	0	<0.055	
2014	11-07	Solid set (pond)	Greens	30	0	<0.055	
Year 2 – 2015 (with Chlorination)							
2015	06-15	Pivot (pond)	Cantaloupe	2	0	<0.055	
2015	06-15	Pivot (pond)	Cantaloupe	2	0	<0.055	
2015	07-02	Pivot (pond)	Cantaloupe	2	0	<0.055	
2015	07-02	Drip (pond)	Tomatoes	8	0	<0.055	
2015	10-19	Drip (pond)	Cucumber	8	–[2]	–[2]	
2015	10-19	Solid set (pond)	Greens	30	–[2]	–[2]	

[1] each row summarizes the results of the five individual produce samples collected on that date

[2] frozen – pending results

Table 7. Statistical comparison of mean *Salmonella* concentrations (MPN/100mL) of water samples collected in 2015 using the chlorination system; (pond) indicates the water source for the irrigation system (with chlorination). Means with the same t Grouping letter are not significantly different. Data were analyzed using an analysis of variance GLM procedure follow by means separation LSD test.

t Grouping	Mean *Salmonella* (MPN/100mL)	N	Sample type
A	0.05857	35	Pond
A	0.02000	8	Pivot (pond)
A	0.01286	35	Spigot/valve (pond)
A	0.00740	25	Drip tape start (pond)
A	0.00640	25	Drip tape end (pond)
A	0.00000	2	Sprinkler

Table 8. Statistical comparison of mean generic *E. coli* concentrations (MPN/100mL) of water samples collected in 2015 using the chlorination system; (pond) indicates the water source for the irrigation system (with chlorination). Means with the same t Grouping letter are not significantly different. Data were analyzed using an analysis of variance GLM procedure follow by means separation LSD test.

t Grouping	Mean generic *E. coli* (MPN/100mL)	N	Sample type
A	5.460	35	Pond
A	5.350	8	Pivot (pond)
A	3.137	35	Spigot/valve (pond)
A	2.044	25	Drip tape end (pond)
A	1.404	25	Drip tape start (pond)
A	0.000	2	Sprinkler

Table 9. Comparison of FDA FSMA agricultural water quality generic *E. coli* thresholds to occurrence of *Salmonella* in water samples collected during the previous CPS-funded project from 2012-2013.

Threshold	*Salmonella* +	*Salmonella* -
Geometric Mean < 126 CFU/100mL	False neg = 42.2%	False pos = 0%
E. coli > GM 126 CFU/100mL	0	0
E. coli < GM 126 CFU/100mL	217	293
STV < 410 CFU/100mL	False neg = 42.3%	False pos = 45.5%
E. coli > 410 CFU/100mL	6	5
E. coli < 410 CFU/100mL	211	288

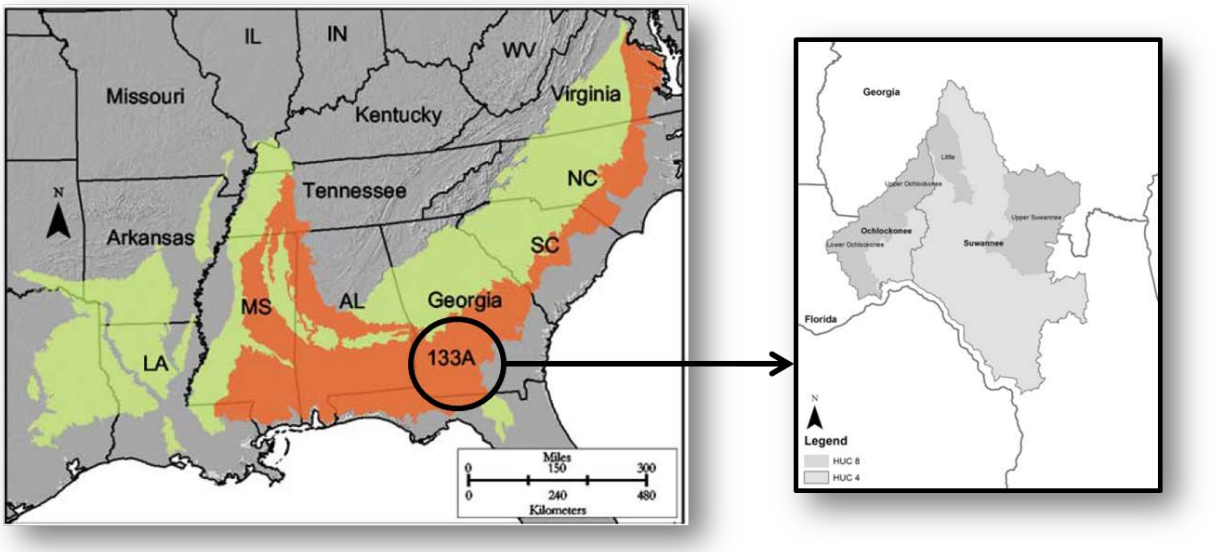


Figure 1. The map on the left shows the Southeastern Coastal Plain USDA NRCS land resource region. CPS-funded projects have been conducted within the watersheds indicated in the map to the right. This project was conducted within the Little River watershed.

Figure 2. Methods used to collect samples from irrigation systems used in the project. From top left in clockwise direction: center pivot irrigation system, pump station valve/spigot, at the end of a drip irrigation system line, and solid set sprinkler system.

Figure 3. Equipment used to collect water samples from near the intake of a pond's pumping station. A multiparameter water quality sonde is used simultaneously to measure water quality parameter in situ.

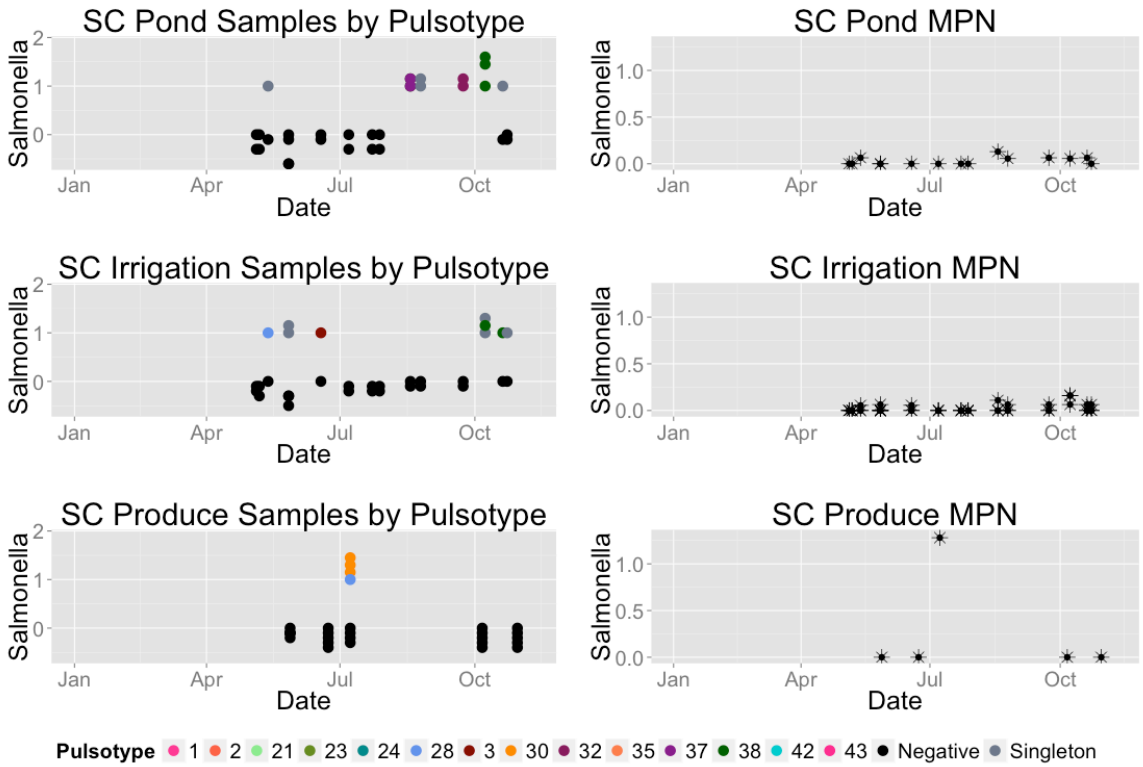


Figure 4. Visualization of the pulsotype data and MPN levels at each pond used for irrigation. Samples were stratified by sample type (source water, irrigation distribution system, produce). Samples that were negative are plotted in black. Positives samples were plotted in varying colors by pulsotype. Unique pulsotypes that were only associated with one isolate are plotted in grey and labeled "Singleton."

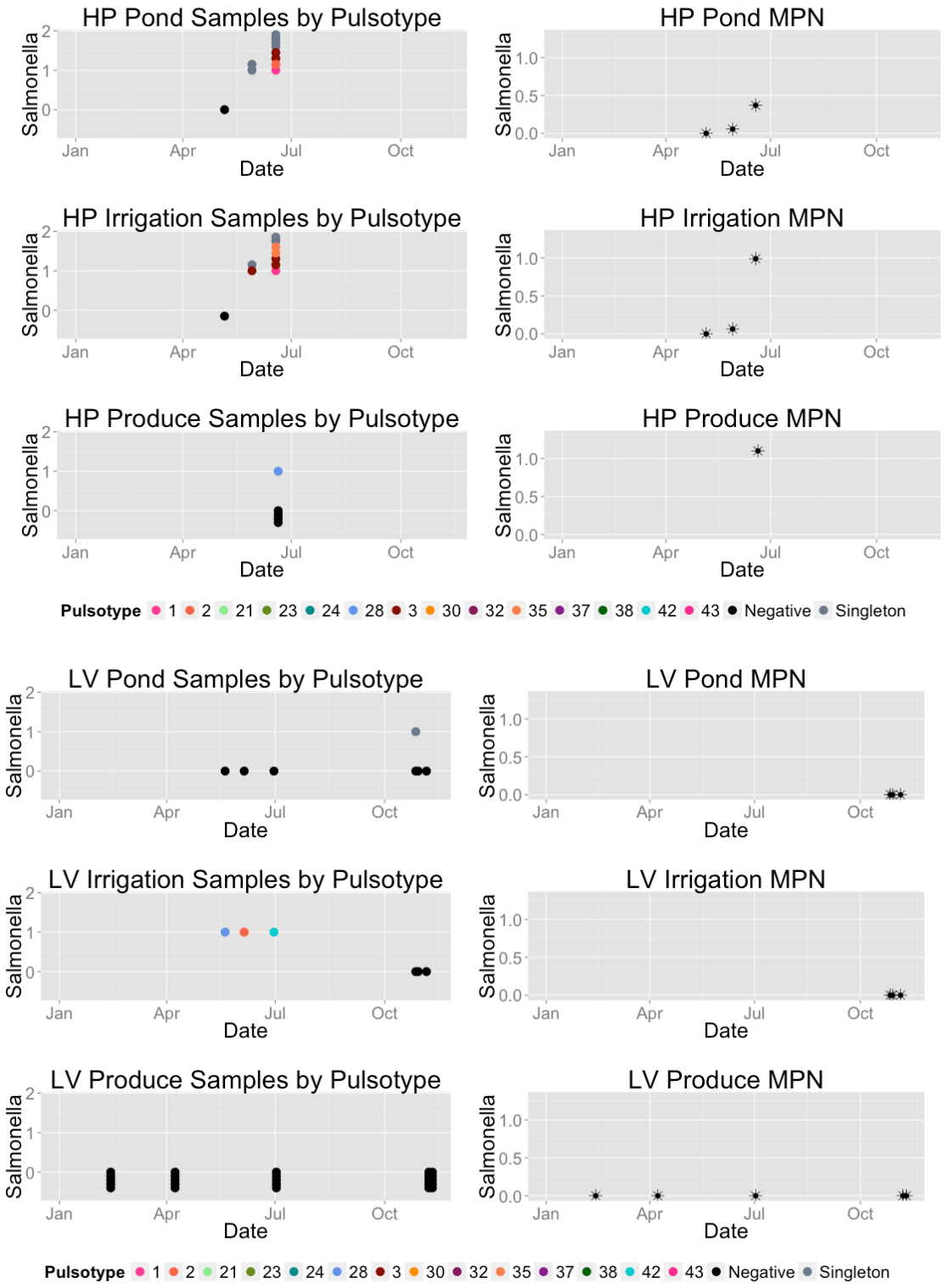
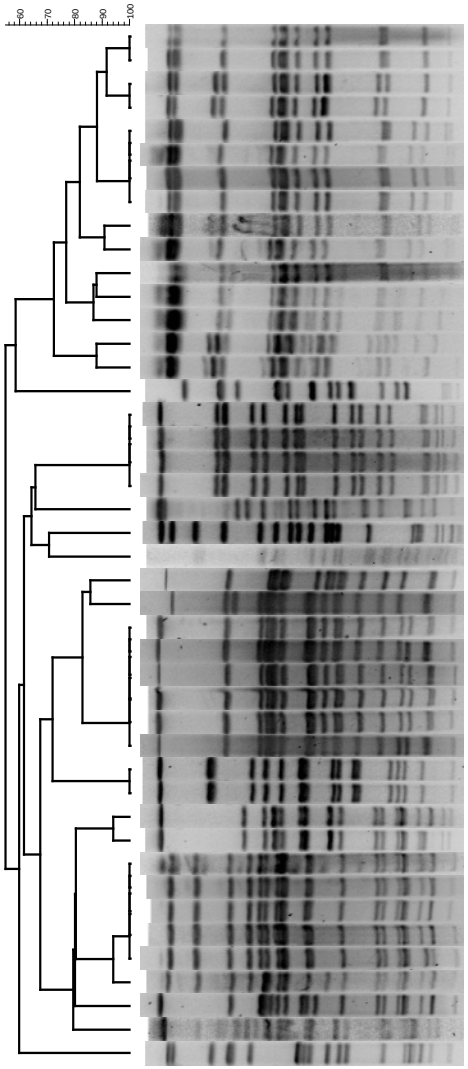


Figure 4 continued. Visualization of the pulsotype data and MPN levels at each pond used for irrigation. Samples were stratified by sample type (source water, irrigation distribution system, produce). Samples that were negative are plotted in black. Positives samples were plotted in varying colors by pulsotype. Unique pulsotypes that were only associated with one isolate are plotted in grey and labeled "Singleton."



UC Davis#	Date	Location	Pond	Pulsotype	Rotation	System	Sample type	Serotype	UGA#
MJ 710(5)	06/18/2014	GA	HP	1	Cantaloupe	Pond	Water	Salmonella Saint Paul	R137 1-3 c
MJ 717(1)	06/05/2014	GA	LV	1	Cantaloupe	Pivot	Water	Salmonella Saint Paul	R133 1-1 a
MJ 710(4)	06/18/2014	GA	HP	2	Cantaloupe	Pond	Water	Salmonella Saint Paul	R137 1-2 a
MJ 718(3)	06/18/2014	GA	HP	2	Cantaloupe	Pivot	Water	Salmonella Saint Paul	R138 1-3 a
MJ 710(3)	06/18/2014	GA	HP	3	Cantaloupe	Pond	Water	Salmonella Saint Paul	R137 1-1 b
MJ 716(2)	05/29/2014	GA	HP	3	Cantaloupe	Pivot	Water	Salmonella Saint Paul	R131 1-1 b
MJ 718(11)	06/18/2014	GA	HP	3	Cantaloupe	Pivot	Water	Salmonella Saint Paul	R138 2-3 a
MJ 719	06/18/2014	GA	SC2	3	Watermelon	Drip Start	Water	Salmonella Saint Paul	R140 2-3 a
MJ 710(2)	06/18/2014	GA	HP	4	Cantaloupe	Pond	Water	Salmonella Saint Paul	R137 2-2 a
MJ 716(1)	05/29/2014	GA	HP	5	Cantaloupe	Pivot	Water	Salmonella Saint Paul	R131 1-1 a
MJ 710(6)	06/18/2014	GA	HP	6	Cantaloupe	Pond	Water	Salmonella Saint Paul	R137 2-2 c
MJ 714(1)	05/27/2014	GA	SC2	7	Watermelon	Drip End	Water	Salmonella Saint Paul	R128 1-3 a
MJ 714(2)	05/27/2014	GA	SC2	8	Watermelon	Drip End	Water	Salmonella Saint Paul	R128 1-3 b
MJ 715(1)	05/29/2014	GA	HP	9	Cantaloupe	Pond	Water	Salmonella Saint Paul	R130 2-1 a
MJ 715(4)	05/29/2014	GA	HP	10	Cantaloupe	Pond	Water	Salmonella Saint Paul	R130 2-1 d
MJ 718(1)	06/18/2014	GA	HP	11	Cantaloupe	Pivot	Water	Salmonella Javiana	R138 1-1 a
MJ 724	08/25/2014	GA	SC2	12	Bell Peppers	Pond Spigot	Water	Salmonella Mbandaka	R164 1-2 b
MJ 729(1)	10/08/2014	GA	SC1	12	Zucchini	Drip Start	Water	Salmonella Mbandaka	R180 1-1 a
MJ 730(1)	10/08/2014	GA	SC1	12	Zucchini	Drip End	Water	Salmonella Mbandaka	R181 1-1 a
MJ 731	10/20/2014	GA	SC1	12	Zucchini	Pond	Water	Salmonella Mbandaka	R182 1-3 a
MJ 710(10)	06/18/2014	GA	HP	13	Cantaloupe	Pond	Water	Salmonella Rubislaw	R137 2-3 c
MJ 723(1)	08/25/2014	GA	SC2	14	Bell Peppers	Pond	Water	III_60:r:e,n,x,z15	R161 2-1 a
MJ 734	10/27/2014	GA	LV	15	Mustard Greens	Pond	Water	Pending	R191 2-2 b
MJ 711(1)	05/13/2014	GA	SC1	16	Squash	Pond	Water	Salmonella Newport	R113 1-3 a
MJ 736(1)	07/08/2014	GA	SC1	17	Cucumber	Rinsate	Cucumber Rinsate	Salmonella Bardo	PW-Cu-E-1-2a
MJ 735	06/20/2014	GA	HP	18	Cantaloupe	Rinsate	Cantaloupe Rinsate	Salmonella sp. Rough "O"	PW-C1-D 3-1 a
MJ 736(2)	07/08/2014	GA	SC1	18	Cucumber	Rinsate	Cucumber Rinsate	Salmonella sp. Rough "O"	PW-Cu-E-1-2b
Positive_1 Emory		GA or FL	unknown	18	Environmental	Pond	Environmental	Salmonella Newport	Positive
Positive_1 UGA	3/2012	GA	unknown	18	Environmental	Pond	Environmental	Salmonella Newport	Positive
Positive_2 UGA	3/2012	GA	unknown	18	Environmental	Pond	Environmental	Salmonella Newport	Positive
Positive_3 UGA		GA	unknown	18	Environmental	Pond	Environmental	Salmonella Newport	Positive
MJ 725	09/23/2014	GA	SC2	19	Bell Peppers	Pond	Water	Salmonella Hartford	R174 1-3 a
MJ 726(1)	09/23/2014	GA	SC2	19	Bell Peppers	Pond Spigot	Water	Salmonella Hartford	R177 1-1 a
MJ 718(10)	06/18/2014	GA	HP	20	Cantaloupe	Pivot	Water	Salmonella Muenchen	R138 2-3 b
MJ 718(4)	06/18/2014	GA	HP	21	Cantaloupe	Pivot	Water	Salmonella Muenchen	R138 2-2 b
MJ 721(1)	08/18/2014	GA	SC2	22	Bell Peppers	Pond	Water	Salmonella Muenchen	R157 1-3 e
MJ 727(1)	10/08/2014	GA	SC1	22	Zucchini	Pond	Water	Salmonella Muenchen	R178 2-3 a
MJ 728(1)	10/08/2014	GA	SC1	22	Zucchini	Pond Spigot	Water	Salmonella Muenchen	R179 1-1 a
MJ 730(4)	10/08/2014	GA	SC1	22	Zucchini	Drip End	Water	Salmonella Muenchen	R181 1-3 a
MJ 732(1)	10/20/2014	GA	SC1	22	Zucchini	Drip End	Water	Pending	R184 1-3 a
MJ 722(1)	08/18/2014	GA	SC2	23	Bell Peppers	Pond Spigot	Water	Salmonella Muenchen	R160 3-1 a
MJ 733(1)	10/23/2014	GA	SC1	24	Zucchini	Drip Start	Water	Salmonella Muenchen	R187 1-1 a
MJ 710(1)	06/18/2014	GA	HP	25	Cantaloupe	Pond	Water	Salmonella Muenchen	R137 1-1 a
MJ 720	06/30/2014	GA	LV	26	Cantaloupe	Pivot	Water	III_16:z10:e,n,x,z15	R144 1-1 a

Figure 5. The dendrogram was constructed using band-band analysis with an optimization of 2.5%, band-matching tolerance at 1.5% followed by UPGMA method for clustering. Twenty-six unique pulsotypes were identified. Some samples had multiple isolates with the same pulsotype, so were considered clones. In this dendrogram, we show only unique pulsotypes from within a given sample.

Figure 6. Four Accu-Tab brand chlorinators were installed at the pumping stations of the irrigation systems withdrawing from ponds (left). Each of the systems required a different number of calcium hypochlorite tablets (right) to achieve a residual free chlorine concentration of 2 mg/L and account for the concentration of suspended solids in the water column.

Figure 7. The cover of the Extension booklet on irrigation water disinfection methods and a summary table of the disinfection methods found on p.3 of the booklet. One hundred copies will be printed for distribution, but the booklet will also be made widely available through online resources at UC Davis and the University of Georgia.

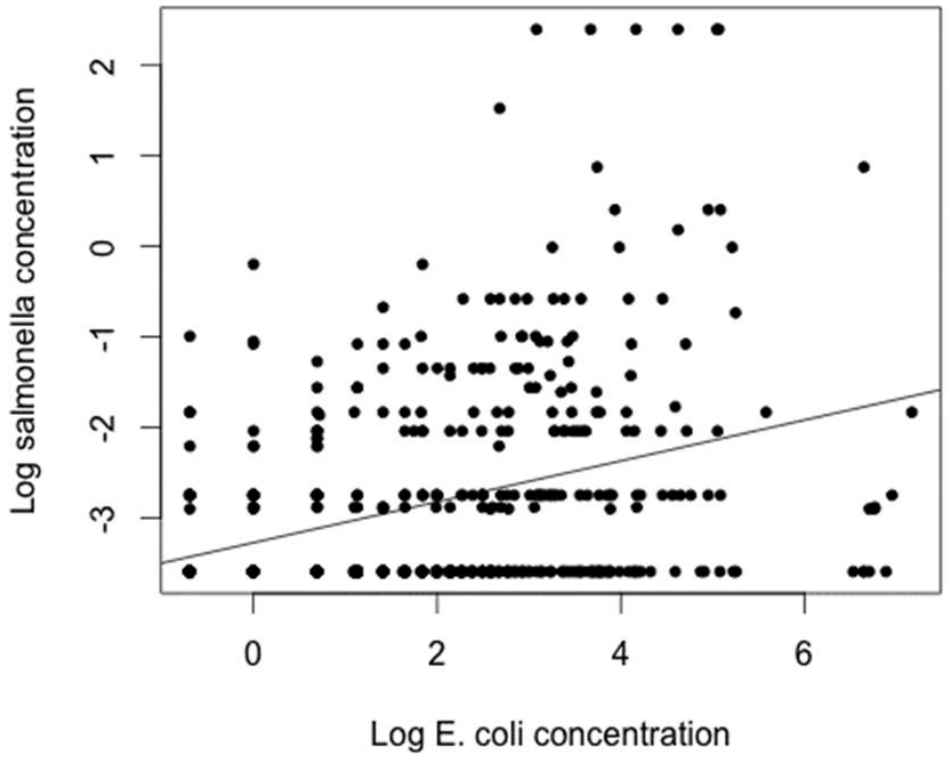


Figure 8. Data from water samples collected in the southeastern Coastal Plain between 2012 – 2013 show poor overall correlation between generic *E. coli* and *Salmonella*.