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Yiru Gu April 15, 2014

The effects of produce type on the concentration and prevalence of microbial contamination of Mexican produce and associated irrigation water

by

Yiru Gu

Juan Leon, PhD, MPH
Advisor

Department of Biology

Juan Leon, PhD, MPH
Advisor

Rustom Antia, PhD
Committee Member

Christopher Beck, PhD
Committee Member

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ABSTRACT

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Foodborne illnesses caused by the consumption of raw contaminated produce represent a significant public health burden. Knowledge regarding how crop type affects contamination is essential to the development of produce-specific intervention strategies to reduce or prevent contamination on the farm. This study investigates the effects of produce type on the concentration and prevalence of fecal contamination by quantifying microbial indicators of fecal contamination (fecal coliforms, *E. coli*, *Enterococcus spp.*, somatic coliphage) on tomatoes, jalapeño peppers, and cantaloupes throughout farm production, as well as in associated surface drip irrigation water from 11 farms in northern Mexico. During the 2011-2012 growing seasons, whole fruit rinses of produce (n=254) were collected during pre-harvest, harvest, distribution, and packing. Water samples (n=76) were collected pre-harvest from the irrigation distribution lines as close as possible to sampled produce. Among produce combined from all production stages, cantaloupes had significantly higher microbial concentrations and were approximately three and over 30 times more likely to be positive for *E. coli* and *Enterococcus*, respectively, compared with other crop types. At each production stage, cantaloupes also had significantly higher microbial concentrations compared with at least one other crop type as well as had higher prevalence of *E. coli* and *Enterococcus* at all production stages except packing. Tomato associated irrigation water had significantly higher *E. coli* concentrations compared with jalapeño and cantaloupe associated water, and was approximately nine and five times more likely to be positive for *E. coli* than were jalapeño and cantaloupe associated water, respectively. Tomato associated water was also over nine times more likely to be positive for somatic coliphage than was cantaloupe associated water. Pre-harvest produce and associated irrigation water were not found to be related in terms of microbial contamination. In general, all produce types had microbial contamination pre-harvest, and both concentrations and prevalence did not differ across the production stages. Because microbial contamination generally did not change from pre-harvest to packing, we recommend the implementation of practices to reduce risk of produce contamination, especially cantaloupe contamination, during pre-harvest.

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INTRODUCTION

Background and importance

Foodborne illness due to consumption of contaminated food is a major public health concern in the United States as well as globally. Of the 48 million Americans estimated to fall ill due to the consumption of contaminated food each year, 128,000 are hospitalized and 3,000 perish (DeWaal and Glassman, 2013). In the past decade, consumption of contaminated raw produce was reported to cause more illnesses (25,222) and outbreaks (696) than any other single-ingredient food. Fruits were linked to 100 outbreaks and 3,629 illnesses, vegetables to 235 outbreaks and 11,839 illnesses, and produce dishes to 361 outbreaks and 9,754 illnesses. Overall, produce accounted for 17% of total foodborne outbreaks and 24% of total illnesses. The costs of these produce-related illnesses amount to up to $39 billion each year for the U.S. (Scharff, 2010).

Several fruits and vegetables, such as cantaloupes, tomatoes, and jalapeño peppers, which are often consumed raw, have been implicated in a large number of high-profile outbreaks in the U.S. and Canada. Most cantaloupe outbreaks have been attributed to *Salmonella*; outbreaks (many of which were multistate) have been attributed to *Salmonella* Chester (Ries *et al.*, 1990), *S.* Poona (CDC, 1991, 2002), *S.* Saphra (Mohle-Boetani *et al.*, 1999), *S.* Oranienburg (Deeks *et al.*, 1998), *S.* Enteritidis (CDC, 2003) and *S.* Litchfield (CDC, 2008c). Cantaloupe outbreaks have also been linked to *E. coli* O157:H7, *Campylobacter* enteritis, and norovirus (CDC, 2003).

Since 1990, consumption of *Salmonella* contaminated raw tomatoes has caused as many as 15 outbreaks in the U.S. (Bartz, 2009). Strains of *Salmonella* that have caused outbreaks include *S.* Javiana (CDC, 2005; Hedberg *et al.*, 1999; Srikantiah *et al.*, 2005), *S.* Montevideo (Hedberg *et al.*, 1999), *S.* Baildon (Cummings *et al.*, 2001), *S.* Newport (CDC, 2007; Greene *et*

al., 2008), *S.* Braenderup (CDC, 2005), and *S.* Typhimurium (CDC, 2007). In 2004, an outbreak occurred consisting of multiple serotypes, including Anatum, Thompson, and Muenchen (CDC, 2005). In 2008, a large multistate outbreak was caused by consumption of jalapeño peppers contaminated with *Salmonella* Saintpaul (CDC, 2008a).

Produce contamination on the farm

Foodborne illness is caused by the consumption of enteric pathogens (e.g. *E. coli* O157:H7, *Salmonella,* norovirus), which are transmitted via the fecal-oral route (Sapers and Doyle, 2009). Pathogen contamination of produce may occur at any point from the farm to the fork, but particular focus has been placed on mechanisms of contamination on the farm. The production process on the farm typically consists of the successive stages: growing, harvesting, distribution, and packing, and contamination can take place at any or multiple stages. Prior to harvest, produce can become contaminated via contact with contaminated soil or water. Soil contamination can occur via the fecal droppings of humans, domestic animals, and wildlife as well as through application of improperly treated fertilizer, manure, and farm effluents. Stormwater runoff can also be sources of soil contamination. Produce that comes into contact with or is grown using irrigation water harboring pathogens can also become contaminated.

During harvest, as produce is being handled, worker health and hygiene can impact produce quality, as workers that are contaminated due to negligent hand-washing, poor farm sanitation, or direct infection, may spread pathogens onto produce (Sapers and Doyle, 2009). Post-harvesting processes pose further risk of contamination due to exposure of produce to equipment surfaces such as conveyer belts, wash tanks, scrub brushes, and packing crates, all of which, if not subject to proper sanitation practices, may harbor pathogens. Furthermore, as

individual produce are packed or washed together towards the final stages of the production process, risk of spread of focal contamination is increased. Previous research has found that contamination for some crop types may increase throughout the production process because of combined risk agents and increased opportunity for cross-contamination (Castillo *et al.*, 2009).

Irrigation water and produce contamination

The quality of irrigation water supplied to produce on the farm is crucial as water can be a source of transmission for harmless microorganisms as well as pathogens. Contaminated irrigation water may pose risk for contamination of the produce to which it is applied, although this is dependent on crop type, crop growing style, system of irrigation (surface, sprinkler, drip), and irrigation frequency (Gerba and Choi, 2009).

Foodborne outbreaks have been attributed to use of contaminated irrigation water. A recurrent U.S. multistate outbreak involving tomatoes was attributed to pond water harboring *Salmonella* Newport that was used to irrigate the crop (Greene *et al.*, 2008). In 2005, an outbreak in Sweden was caused by the consumption of lettuce irrigated by a stream contaminated with verotoxin-producing *E. coli* O157:H7 (Soderstrom *et al.*, 2008). In 2006, shredded lettuce that had been irrigated with well water unintentionally contaminated with *E. coli* O157:H7 caused a U.S. outbreak (FDA, 2008). In 2008, irrigation water contaminated with *Salmonella* Saintpaul caused a U.S. outbreak of jalapeño and Serrano peppers (CDC, 2008b).

Quantification of produce and water contamination

Quantifying pathogen contamination is difficult due to low concentrations, low prevalence, and focal distribution of pathogens in the environment. Because the source of enteric pathogens originates from feces of humans and warm-blooded animals, quantifying fecal

contamination provides an estimation of the likelihood of pathogen contamination. Thus, microbial indicators of fecal contamination are often quantified, as these microorganisms are frequently excreted from intestinal tracts of humans and warm-blooded animals in large quantities, and thus are easily detected in the environment (Tyagi *et al.*, 2006).

Numerous criteria exist for choosing a suitable indicator. Important criteria include that the ideal indicator be present when enteric pathogens are present and absent when the pathogens are absent (Ray, 2003). Furthermore, there should be a direct correlation between the amount of the indicator present and the likelihood of a pathogen(s) being present. The ideal indicator should be similar to pathogens in growth, survival, and resistance, and should not grow slower or perish faster than pathogens. Moreover, the indicator should not be able to multiply outside the host (Payment and Locas, 2011). Finally, the indicator should allow easy detection in the laboratory. Because no single indicator fulfills all such criteria, measuring a suite of indicators rather than a single indicator is recommended (Tyagi *et al.*, 2006).

Common bacterial indicators include *E. coli* and *Enterococcus spp*. as well as fecal coliform bacteria, a subset of all fecal bacteria, which provides a larger range of detection of fecal contamination than just one indicator species alone. Fecal coliforms consist of mostly *E. coli,* as well as *Klebsiella* and *Enterobacter spp*. (Ray, 2003). Somatic coliphage is often used as a proxy for enteric viruses, as the two are related in terms of structure, transport, and survival in the environment (Gerba, 1987). Specifically, Ballester *et al.* (2005) discovered that the presence of somatic coliphage, and not of fecal bacteria, was significantly associated with the presence of enteric viruses. Studies done on indicator-pathogen relationships in water have reported both significant (Payment *et al.*, 2000) and insignificant correlations (Lemarchand and Lebaron, 2003;

Lipp *et al.*, 2001). Other studies have found a mixture of both (Morinigo *et al.*, 1990; Horman *et al.*, 2004).

Research goal

Understanding the effects of produce type on risk of pathogen contamination is essential as each type of produce has unique physical, chemical, and biological characteristics that may affect its level and prevalence of contamination (Beuchat, 2002). Cantaloupes have a rough and netted external rind, whereas tomatoes and jalapeños have smooth, waxy surfaces (Castillo *et al.*, 2009). Such differences may influence microbial attachment to and detachment from each crop type and provide different ecological niches for different microorganisms. Knowledge regarding the effects of produce type on microbial contamination may be important in designing produce-specific intervention strategies to reduce or prevent contamination.

The goal of this study was to determine the effects of produce type on the concentration and prevalence of fecal contamination by quantifying microbial indicators of fecal contamination (fecal coliforms, generic *E. coli*, *Enterococcus spp.*, somatic coliphage) on tomatoes, jalapeño peppers, and cantaloupes throughout farm production, as well as in associated surface drip irrigation water from 11 farms in northern Mexico during the 2011-2012 growing seasons. Based on previous findings, we hypothesized that cantaloupes would be linked to greater concentrations and prevalence of contamination compared with tomatoes and jalapeño peppers. Because all farms used well water for irrigation and used surface drip irrigation, no differences in irrigation water across crop types were expected.

METHODS

Sample collection

This study was approved by the institutional review board of Emory University (IRB00035460). From May to December in 2011 and 2012, produce and irrigation water samples were collected from 11 farms within the states of Nuevo León and Coahuila in Mexico. Five farms produced cantaloupes, five farms produced tomatoes, and five farms produced jalapeños, with four farms producing both tomatoes and jalapeños.

Produce samples were collected at four successive steps in the production process: before harvest, during harvest, during distribution away from the field, and at the packing shed, if present. At each step, triplicate produce samples were collected at random locations in the field (before and during harvest), truck (during distribution), or packing shed, and composited. Composite samples represented whole fruit rinses of 54 tomatoes, 42 jalapeños, or 6 cantaloupes in 1500 ml of 0.15% sterile peptone water. The specific numbers of tomatoes, jalapeños, and cantaloupes used in each rinse was chosen to provide an equivalent surface area across produce types (736 cm^2 of fruit per ml). Rinses were done in Whirl-Pak bags (Nasco, Fort Atkinson, WI), in which produce were shaken for 30 seconds, massaged for 30 seconds, and then shaken again for 30 seconds.

Irrigation water samples (1.5 L) were collected pre-harvest from irrigation lines in the field. When able, water samples were collected from the drip tape hose connection as close as possible to the location of the matched sampled produce. Otherwise, samples were collected from the main distribution line to the field running perpendicular to the rows. Triplicate water samples were composited for a total of 4.5 L. All samples were stored in coolers on ice packs during transport and were refrigerated at 4°C upon arrival at the laboratory at the Universidad

Autónoma de Nuevo León. All samples were kept at 4°C until microbial analysis, which generally took place within 1 to 4 days after arrival.

Microbial analysis

Composite samples were partitioned into smaller subsamples for microbial indicator testing. For bacterial indicator analyses, samples were concentrated by membrane filtration. Sample volumes, ranging from 10 µl to 50 ml for produce and from 10 to 250 ml for water were vacuum filtered through a 47 mm, 0.45 µm pore size S-Pack filter (Millipore, Billerica, MA). Following filtration, filters were placed on selective media for microbial quantification. *Enterococcus spp.* were enumerated using KF Streptococcus agar (Oxoid Limited, Basingstoke, Hampshire, UK) incubated at 37°C for 48 hours. *E. coli* and fecal coliforms were enumerated on RAPID'E. coli 2 agar (Bio-Rad Laboratories, Inc., Hercules, CA) incubated at 44°C for 24 hours.

Somatic coliphage was screened using FastPhage MPN Quanti-tray (Charm Sciences, Inc., Lawrence, MA) incubated at 37°C for 6 hours. Samples were mixed with fluorescence-based media inoculated with *E. coli* and then partitioned into Most Probable Number (MPN) compartments. Because compartments with at least one plaque forming unit (PFU) fluoresce under UV light, the number of fluorescing compartments was used to determine MPN using a conversion table (Charm Sciences, 2010). Depending on the concentration of particulates in the original sample, 100 ml of sample or 10 ml of sample diluted with 90 ml of 0.15% peptone water was used for analysis.

Microbial quantification

The number of colony forming units (CFU) per filtered volume was used to quantify bacterial indicator concentrations (*E. coli*, *Enterococcus*, fecal coliforms) in each sample. The most probable number (MPN) of plaque forming units was used to quantify somatic coilphage. Indicator concentrations in produce samples were measured in CFU or MPN per fruit and in CFU or MPN per ml. Measuring concentrations per ml, each ml of which was equivalent to 736 cm^2 of rinsed fruit surface, served to correct for differences in fruit surface area among crop types. Indicator concentrations in irrigation water samples were measured in CFU or MPN per 100 ml to enable comparison to U.S. Environmental Protection Agency Recreational Water Quality standards (EPA, 2012).

The limit of detection of the microbial assays was 1 CFU per largest effective volume and 1 MPN per 100 ml. The limit of quantification was 250 CFU per smallest effective volume and 2420 MPN per 100 ml. In order to calculate the mean concentration of each indicator in a given sample across replicate assays, the quantifiable range for CFU was designated as 25 to 250 CFU per plate (Table 1). For samples with CFU values that fell within the quantifiable range (type 3), an arithmetic mean of these values and their corresponding sample volumes was calculated. For some samples, CFU values from all plates were outside of this range; therefore, the concentration of indicators in these samples was estimated or imputed.

Indicator concentrations were estimated when CFU data were available, but values were outside the range of 25-250. For samples with all CFU values below 25, data from the assays using the largest effective volumes were used for estimated indicator concentrations (Table 1). This approach was also used to estimate indicator concentrations from samples with CFU values

both above and below, but not within the quantifiable range. Values from assays with the smallest effective volume were used to estimate indicator concentrations from samples that had all CFU values above 250.

Indicator concentrations were imputed when CFU data were not available. In cases where all CFU values were zero, a value of half the limit of detection was imputed (0.5 CFU divided by the maximum effective volume assayed; Table 1) (Shumway *et al.*, 1989). In cases where all CFU were too numerous to count (TNTC), a value of twice the upper limit of quantification was imputed (500 CFU divided by the minimum effective volume assayed) (Shumway *et al.*, 1989). In odd cases where all CFU values were either 0 or TNTC, a value of twice the upper limit of quantification was imputed.

For statistical purposes, all samples (types 1-7) were used for analysis (Table 1). Statistical analyses conducted using only samples within the quantifiable range (type 3) and analyses conducted using all sample types produced the same results (data not shown). At times, statistical analyses could not be run using only type 3 samples, due to small sample size. Thus, it was advantageous to consider all samples.

Statistical analysis

Descriptive statistics

Statistical analyses were conducted using JMP Pro 10 (SAS Institute Inc., Cary, NC) at an alpha level of 0.05. However, descriptive statistics, including geometric means, confidence intervals, and indicator prevalence, were calculated using SAS 9.3 (SAS Institute Inc., Cary, NC). Because of the large variation in microbial concentrations, geometric means rather than arithmetic means were used to provide means that would be less biased toward exceptionally

high or low counts (FDA, 2013; Mostert and Jooste, 2002). Indicator concentrations were normalized using $\log_{10}$ transformation. The Shapiro-Wilk test assessed that data distributions were not normal after transformation (Shapiro and Wilk, 1965). Thus, non-parametric tests were used for statistical analyses. Analyses of data from produce were conducted twice, once using concentration units of CFU per fruit and again using CFU per ml.

Inferential statistics

The Kruskal-Wallis test was conducted to determine whether one or more differences in mean rank indicator concentrations existed between or among produce types (Kruskal, 1952). If such differences existed, the Steel-Dwass All Pairs test was conducted post-hoc to determine specific pairwise comparisons (Steel, 1959; Critchlow and Fligner, 1991; Dwass, 1960).

Logistic models were constructed to provide quantitative estimates (odds ratios) of the differences in indicator prevalence between produce types for produce samples combined from all production stages and for irrigation water samples. Logistic models quantified association between indicator prevalence on produce or water (outcome) and produce type (predictor). For cases in which inadequate sample size or 100% prevalence prohibited odds ratio calculations, Fisher's 2x2 Test was conducted (Fisher, 1922).

For produce samples separated by production stage, Fisher's Exact (2x3) Test was first used to detect whether at least one significant difference in indicator prevalence existed between or among produce types. For such instances, Fisher's 2x2 Test was then conducted to reveal specific pairwise differences. Odds ratios could not be calculated for all the prevalence comparisons among produce samples separated by production stage because produce types often had 100% prevalence, and so were not included. For odds ratios and Fisher's 2x2 pairwise

comparisons, the Bonferroni approach was used to correct alpha according to the number of groups being compared (Bonferroni, 1936; Miller, 1981).

Spearman's rank correlation coefficients were calculated to examine possible correlations in microbial concentrations between produce associated irrigation water and pre-harvest produce type (Spearman, 2010). Odds ratios were calculated to determine association between irrigation water and produce in terms of microbial prevalence.

Power analysis

Power analyses were conducted to determine the required sample sizes to detect existing differences in mean microbial concentrations (Dean, 2013) or microbial prevalence (Pezzullo, 2009) between produce types at each production stage for future field studies. OpenEpi Version 3.01 was used for the analysis of sample sizes required to detect differences in mean microbial concentrations (Dean, 2013; Rosner, 2000). Specifically, at each production stage, for each indicator, the sample sizes required for both produce types were calculated using the $\log_{10}$ transformed arithmetic mean difference in microbial concentrations between the produce types and their respective standard deviations (Fitts, 2011). Analyses were only conducted for pairwise comparisons that were not found to be significant in terms of differences in microbial concentrations by the Steel-Dwass All Pairs test.

Additionally, a power analysis was conducted to determine the required sample sizes to detect existing differences in microbial prevalence (Pezzullo, 2009) between produce types at each production stage. Such analyses were only conducted for instances in which no significant difference in microbial prevalence was detected between produce types using Fisher's 2x2 Test. Specifically, at each production stage, for each indicator, the sample sizes required for both

produce types were calculated using the observed prevalence of each produce type (Pezzullo, 2009). Calculations for both power analyses were based on an alpha level of 0.05, 80% power, and equal sample sizes of each group.

RESULTS

Descriptive statistics

The Shapiro-Wilk test revealed non-normal distributions of indicator concentrations on produce samples among all produce types (Figure 1). Regarding irrigation water samples, half of the distributions of indicator concentrations on produce associated water were also non-normal (Figure 2). Therefore, non-parametric tests (Kruskal-Wallis and Steel-Dwass All Pairs) were employed for statistical analyses.

In some instances, geometric mean indicator concentrations fell below the limit of detection or exceeded the limit of quantification (Tables 2, 3-5). This occurred when a large proportion of samples had microbial assays of types 1, 6, or 7, and were thus assigned corresponding estimated values that fell below the limit of detection or surpassed the limit of quantification (Table 1).

Comparisons of microbial concentrations

Indicator concentrations between and among produce types were statistically compared, with geometric mean indicator concentrations used to provide a quantitative estimate of such differences. Among produce combined from all production stages, cantaloupes had significantly higher indicator concentrations when compared to jalapeños and tomatoes, regardless of indicator type ($p<0.0001$*; Table 3). Specifically, geometric mean fecal coliform concentrations on cantaloupes were 2.61 and 1.90 $\log_{10}$ CFU/fruit higher than those on jalapeños and tomatoes, respectively. Geometric mean *E. coli* concentrations on cantaloupes were 2.56 and 2.64 $\log_{10}$ CFU/fruit higher, *Enterococcus* concentrations were 3.62 and 3.7 $\log_{10}$ CFU/fruit higher, and

somatic coliphage concentrations were 2.42 and 2.73 $\log_{10}$ MPN/fruit higher than those on jalapeños and tomatoes, respectively.

The same trend of significant differences in indicator concentrations across produce types was found for produce samples measured in units of $\log_{10}$ CFU or MPN/ml ($p<0.0001$*; Table 3). Such statistical comparisons accounted for differences in surface area among the different crops by measuring indicator concentrations in each ml of sample, which contained rinses of 736 cm^2 of fruit surface area. The finding that cantaloupes had significantly higher indicator concentrations both when surface area was accounted and unaccounted for, suggests that cantaloupes did not exclusively have higher indicator concentrations because of larger surface area. However, when adjusted for differences in fruit surface area, the differences in concentrations on cantaloupes compared to that on the other produce types were smaller in magnitude than the same differences when measured per fruit.

Specifically, geometric mean fecal coliform concentrations on cantaloupes were 1.76 and 0.95 $\log_{10}$ CFU/ml higher than those on jalapeños and tomatoes, respectively (Table 3). Geometric mean *E. coli* concentrations on cantaloupes were 1.71 and 1.68 $\log_{10}$ CFU/ml higher, *Enterococcus* concentrations were 2.78 and 2.74 $\log_{10}$ CFU/ml higher, and somatic coliphage concentrations were 1.57 and 1.77 $\log_{10}$ MPN/ml higher than those on jalapeños and tomatoes, respectively. Lastly, all produce samples measured in ml also had lower geometric means than counterpart samples measured in fruit.

In general, among produce samples combined from all production stages, there were no significant differences in indicator concentrations between jalapeños and tomatoes, regardless of units measured (Table 3). However, in one instance, tomatoes had significantly higher fecal

coliform concentrations than did jalapeños when measured in ml, with a geometric mean difference of 0.81 $\log_{10}$ CFU/ml ($p<0.0001$*). In another instance, jalapeños had significantly higher *E. coli* concentrations than did tomatoes when measured in fruit, with a geometric mean difference of 0.08 $\log_{10}$ CFU/fruit ($p<0.0001$*).

Microbial concentrations were also statistically compared across produce types at each stage of production, to examine whether trends in contamination across produce types varied as produce moved through the production process. Specifically, among produce measured in fruit, during pre-harvest, harvest, and distribution, for all indicators, cantaloupes had significantly higher microbial concentrations compared with jalapeños and tomatoes ($P<0.05$; Table 4). At the packing shed, for all indicators, cantaloupes had significantly higher microbial concentrations compared with tomatoes ($P<0.05$) as well as significantly higher somatic coliphage concentrations compared with jalapeños ($p=0.0036$*).

In general, there were only a few significant differences in indicator concentrations between jalapeños and tomatoes at each production stage (Table 4). For example, during pre-harvest, harvest, and distribution, jalapeños had significantly higher *E. coli* concentrations compared with tomatoes ($p<0.0001$*). Furthermore, jalapeños also had significantly higher somatic coliphage concentrations compared with tomatoes at pre-harvest ($p<0.0001$*). Overall, we found that when produce samples were separated by production stage, cantaloupes had significantly higher microbial concentrations compared with at least one produce type at each production stage.

Such statistical comparisons repeated with produce samples measured in ml rendered similar results, except that no significant differences in fecal coliform concentrations during

harvest (p=0.1143) or in somatic coliphage concentrations at the packing shed (p=0.1145) across produce types were detected (Table 5). Additionally, cantaloupes had significantly higher fecal coliform concentrations only when compared with jalapeños at pre-harvest (p=0.0180*) and not with tomatoes. Lastly, when produce samples were measured in ml, there were no significant differences in microbial concentrations between jalapeños and tomatoes for any indicator at any production stage.

Among irrigation water samples, the only significant difference in indicator concentrations among produce types was found in *E. coli* concentrations (p=0.0062*; Table 3). *E. coli* concentrations in tomato associated water were significantly higher than those in cantaloupe and jalapeño associated water (p=0.0062*). Specifically, geometric mean *E. coli* concentrations in tomato associated water were 0.49 and 0.82 $\log_{10}$ CFU/100 ml higher than those in cantaloupe and jalapeño associated water, respectively.

In summary, among produce samples, cantaloupes had significantly higher microbial concentrations compared with jalapeños and tomatoes for all indicators at pre-harvest, harvest, and distribution. During packing, cantaloupes also had significantly higher microbial concentrations compared with tomatoes for all indicators. In general, there were no significant differences in microbial concentrations between jalapeños and tomatoes. Among produce associated irrigation water samples, there were no significant differences in indicator concentrations among produce types, except for *E. coli*, of which tomato associated water had significantly higher concentrations compared with cantaloupe and jalapeño associated water.

Comparisons of microbial prevalence

Among produce samples combined from all production stages as well as irrigation water samples, odds ratios were used for statistical comparisons of indicator prevalence between produce pairs by providing a quantitative estimate of the likelihood of contamination of one produce type compared to another (Table 6). Odds ratios were unable to be calculated for comparisons of fecal coliform prevalence due to inadequate sample sizes; specifically, there were either no or too few negative samples.

Among produce samples combined from all production stages, cantaloupes were over three and a half times more likely to be positive for *E. coli* than were jalapeños (p=0.0001*), with a 25% greater occurrence of contamination (Table 6). Moreover, cantaloupes were nearly three times more likely to be contaminated with *E. coli* than were tomatoes (p=0.0004*), having a 22% greater prevalence. Regarding *Enterococcus*, cantaloupes were over 44 times more likely to be contaminated with the indicator than were jalapeños (p<0.0001*) and over 30 times more likely compared with tomatoes (p<0.0001*). Specifically, cantaloupes had a 29% and a 22% greater prevalence of *Enterococcus* contamination than that of jalapeños and tomatoes, respectively. Lastly, cantaloupes also had a significantly higher prevalence of fecal coliforms compared with jalapeños (8% difference; p=0.0058*).

Microbial prevalence was also statistically compared across produce types using produce samples separated by production stage (Table 7). At pre-harvest, a significant difference in *E. coli* (p=0.0360*), *Enterococcus* (p=0.0002*), and somatic coliphage (p=0.0383*) prevalence was detected across produce types. Specifically, at pre-harvest, 41% of cantaloupes were positive with *E. coli*, compared with only 14% of jalapeños and 15% of tomatoes. Furthermore, at pre-

harvest, cantaloupes had significantly higher prevalence of *Enterococcus* compared with jalapeños (33% difference; p=0.0004*) and tomatoes (27% difference; p=0.0012*). However, at pre-harvest, 100% of jalapeños were positive for somatic coliphage, followed by 93% of cantaloupes and 75% of tomatoes.

During harvest, a significant difference in *E. coli* (p=0.0315*; Table 7) and *Enterococcus* (p=0.0032*) prevalence was detected across produce types. Specifically, during harvest, 29% of cantaloupes were positive with *E. coli*, compared with 5% of jalapeños and 8% of tomatoes. Additionally, during harvest, cantaloupes had significantly higher prevalence of *Enterococcus* compared with jalapeños (24% difference; p=0.0041*). During distribution, a significant difference in *Enterococcus* prevalence (p=0.0457*) was detected across produce types, such that 96% of cantaloupes were positive compared with 80% of tomatoes and 65% of jalapeños. In summary, at pre-harvest and harvest, cantaloupes had higher prevalence of *E. coli* compared with jalapeños and tomatoes, and also had higher prevalence of *Enterococcus* compared with the other produce types at all production stages except packing.

Among irrigation water samples, tomato associated water was over nine times more likely to be contaminated with *E. coli* (p=0.0007*) than was jalapeño associated water, with a 50% greater prevalence (Table 8). Tomato associated water was also nearly five times more likely to be positive for *E. coli* than was cantaloupe associated water (p=0.0012*), with a 38% greater prevalence. Furthermore, tomato associated water was over nine times more likely to be contaminated with coliphage than was cantaloupe associated water (p=0.0017*), with a 50% greater prevalence.

In summary, among produce combined from all production stages, cantaloupes were significantly more likely to be positive for *E. coli* and *Enterococcus* than were jalapeños and tomatoes. At all production stages except packing, cantaloupes had higher prevalence of certain indicators compared with at least one other produce type. Among irrigation water, tomato associated water was significantly more likely to be positive for *E. coli* than were jalapeño and cantaloupe associated water. Tomato associated water was also significantly more likely to be positive for coliphage than was cantaloupe associated water.

Microbial contamination on produce through farm production

Overall, as each crop type moved through the production process, microbial concentrations and prevalence did not change. In general, samples of each produce type from the different production stages did not have substantially different geometric mean microbial concentrations (Tables 4, 5) based on overlapping 95% CIs. Specifically, for each indicator, the initial microbial concentrations that produce harbored beginning at pre-harvest were not considerably different, based on overlapping 95% CIs, from those on produce collected at any of the subsequent production stages. Such a pattern was observed for each produce type, and in general, for all indicators. Similarly, samples of each produce type for each indicator from the different production stages did not have considerable differences in microbial prevalence (Table 7).

Correlations and associations between pre-harvest produce and irrigation water

Possible relationships between pre-harvest produce types and associated irrigation water in terms of microbial concentrations and prevalence were examined. In general, there were no significant correlations between pre-harvest produce and matched irrigation water in terms of

microbial concentrations (Table 9). However, a significant negative correlation in *E. coli* concentrations was detected between pre-harvest cantaloupes and associated irrigation water (ρ= -0.3714; p=0.0236*). There were no significant associations between produce and irrigation water in terms of microbial prevalence (Table 10). Overall, there were no major correlations or associations between produce samples and irrigation water samples in terms of microbial contamination.

Power analysis

For all pairwise comparisons of microbial concentrations that were not found to be significant by the Steel-Dwass test (Table 4), a power analysis was conducted to determine whether the number of samples in our study were sufficient to detect such existing differences in mean microbial concentrations between produce types at each production stage for each indicator (Tables 11, 12). These sample sizes were calculated to inform the appropriate sample sizes to detect meaningful differences for future studies. The analysis revealed that for all the pairwise comparisons in question, for future studies, we required a range of sample sizes: fecal coliforms (37 to 122), *E. coli* (6 to 45), *Enterococcus* (9 to 800), and somatic coliphage (91 to 33,241) (Table 12).

Similarly for all pairwise comparisons of microbial prevalence that were not found to be significant by Fisher's 2x2 Test (Table 7), a power analysis was conducted to determine the required sample sizes to detect existing differences in microbial prevalence between produce types at each production stage for each indicator (Table 13). These sample sizes were calculated to inform the appropriate sample sizes to detect meaningful differences for future studies. The analysis revealed that for all the pairwise comparisons in question, for future studies, we required

a range of sample sizes: fecal coliforms (73 to 283), *E. coli* (11 to 19,460), *Enterococcus* (16 to 915), and somatic coliphage (26 to 1509) (Table 13).

DISCUSSION

The purpose of this study was to determine the effects of produce type on concentration and prevalence of fecal contamination by quantifying fecal coliforms, *E. coli*, *Enterococcus spp.*, and somatic coliphage on tomatoes, jalapeño peppers, and cantaloupes throughout farm production, as well as in associated irrigation water from 11 Mexican farms.

Cantaloupes have greater contamination compared to jalapeños and tomatoes

In general, this study found that throughout farm production, cantaloupes had higher microbial concentrations and prevalence compared with jalapeños and tomatoes. Specifically, cantaloupes had higher microbial concentrations compared with jalapeños and tomatoes during pre-harvest, harvest, and distribution, and in some instances, during packing. Overall, cantaloupes on the farm were more likely to be contaminated than were jalapeños and tomatoes and had higher microbial prevalence than the other produce types during pre-harvest, harvest, and distribution.

Previous farm studies have also reported higher and more frequent microbial contamination of cantaloupes in comparison with other crop types. A study done in Texan farms found that, among produce samples collected in the field, 13.0% of cantaloupes were positive for *E. coli* at a limit of detection of 1.4 $\log_{10}$ CFU per cantaloupe, while oranges and parsley had 0% and 1.0% prevalence, respectively, at a limit of detection of 1.4 $\log_{10}$ CFU per orange and 0.6 $\log_{10}$ CFU per gram of parsley. Among produce samples collected in the packing shed, 21% of cantaloupes were positive for *E. coli*, compared with 6.0% of oranges and 3.0% of parsley (Duffy *et al.*, 2005).

Another study done in farms and packing sheds in southern U.S. found the mean *E. coli* concentration of 1.2 ± 0.10 $\log_{10}$ CFU/g on cantaloupes to be significantly higher compared with celery, collards, parsley, spinach, and turnip greens (Ailes *et al.*, 2008). The researchers also found that the mean *Enterococcus* concentration on cantaloupes (4.1± 0.09 $\log_{10}$ CFU/g) was significantly higher than those on arugula, cabbage, celery, cilantro, collards, dill, kale, parsley, spinach, Swiss chard, and turnip greens. Furthermore, Ailes *et al.* (2008) found the prevalence of *E. coli* on cantaloupes (25%) to be significantly higher compared with collards, dill, spinach, and turnip greens, all of which tested negative for *E. coli* at a limit of detection of 0.70 $\log_{10}$ CFU/g. The researchers also found the prevalence of *Enterococcus* on cantaloupes (100%) to be significantly higher compared with arugula, celery, cilantro, collards, kale, and turnip greens.

Proposed mechanisms for greater contamination of cantaloupes

Many factors may affect how susceptible produce are to microbial adherence and growth. Factors include morphology and topography of plant surfaces, plant health, internal composition, metabolic activity, and native microflora (Beuchat, 2002). There are various explanations for why the surface of cantaloupes may harbor higher indicator concentrations compared to the surfaces of other produce types such as tomatoes and jalapeños.

Cantaloupes have a unique netted rind which may support microbial binding and impede detachment (Castillo *et al.*, 2009). Cantaloupes are rich in sugar content (Golden *et al.*, 1993) and have low acidity and high water activity (0.97 to 0.99), all of which may support microbial growth (Bhagwat, 2006). Furthermore, cantaloupes are grown in direct contact with soil, which provides additional opportunities for cross-contamination. Lastly, the larger size of cantaloupes may require more handling by farmworkers, during which cross-contamination is possible.

Unique surface structure

The outer surface of cantaloupes may support bacterial attachment, survival, and growth while inhibiting removal (Castillo *et al.*, 2009). The rind of cantaloupes is covered by rough and porous netting, comprised of cracked hydrophobic cuticle. Such structure with rifts and micropockets increases surface area to which microorganisms may bind and protects microorganisms from detaching. The rinds also shield attached microorganisms from sunlight, washing, and antimicrobial agents, and protect from desiccation. Biofilm formation may also be facilitated.

Research has demonstrated the survival and growth of pathogens or microorganisms on cantaloupe rinds. Stine *et al.* (2005a) found that *E. coli* O157:H7, *E. coli* ATCC 25922, coliphage PRD-1, *Shigella sonnei*, *Clostridium perfringens*, and hepatitis A virus persisted significantly longer on cantaloupes than on lettuce and bell peppers during pre-harvest. Del-Rosario and Beuchat (1995) reported increasing levels of *E. coli* 0157:H7 on cantaloupe rinds for four days after inoculation at 25°C. Annous *et al.* (2004) found that *S.* Poona inoculated on cantaloupe rinds increased at room temperature for up to 3 days, with other researchers reporting similar results (Beuchat and Scouten, 2004; Richards and Beuchat, 2004). In a separate study, Annous *et al.* (2005) also found that *S.* Poona and *Salmonella* Michigan rapidly established biofilms on cantaloupe rinds after inoculation at room temperature.

Studies have also demonstrated the difficulty of microbial detachment from cantaloupe rinds. Ukuku and Sapers (2001) discovered that *Salmonella* inoculated onto cantaloupe rinds were not significantly reduced by water washing. Furthermore, Parnell *et al.* (2005) found that *Salmonella* detached more frequently from honeydew melons, which have smooth, unnetted

rinds, than from cantaloupes after submersion in water for 60 seconds. Similarly, Park and Beuchat (1999) reported lower levels of *Salmonella* and *E. coli O157:H7* on honeydew melons than on cantaloupes after scrubbing rinds for 3 minutes with water. Similar results were found by Ukuku and Fett (2002b), such that water washing did not reduce *Salmonella* concentrations on cantaloupe rinds.

The external surfaces of jalapeños and tomatoes are covered by a smooth, waxy cuticle that may better impede microbial adherence and growth and make for easier detachment than the rinds of cantaloupes. Castro-Rosas *et al.* (2011) observed a decline in *Salmonella* and *E. coli* spot inoculated onto jalapeños within 24 hours at both 3 and 25°C. Liao *et al.* (2010) found rapid increase of *Salmonella* Saintpaul dip inoculated onto jalapeños at 20°C, but detected less than 10% of the pathogen on the edible pod and most in the stem and calyx region of the pepper. This suggests that the hydrophobic surface of the pod may be less favorable for microbial colonization compared with the rougher surfaces of the stem and calyx. If such were the case, it would also explain the discrepancy of the findings of Castro-Rosas *et al.* (2011) and Liao *et al.* (2010), as the dip method inoculates the entire pepper, including stem and calyx, whereas spot inoculates only the pod surface.

Likewise, Ma *et al.* (2010) reported lack of growth of *Salmonella* spot inoculated on both tomatoes and jalapeños at 4, 12, and 21°C. Other studies have similarly observed that *Salmonella* either survives or declines slowly on tomato surfaces (Allen *et al.*, 2005; Beuchat and Mann, 2008; Das *et al.*, 2006; Drosinos *et al.*, 2000; Guo *et al.*, 2002; Wei *et al.*, 1995). Zhuang *et al.* (1995), however, found *Salmonella* to grow rapidly when dip inoculated onto tomatoes at 30°C, as well as to grow within 7 days at 20°C. This again may be attributed to the fact that the dip method additionally inoculates stem scar tissue, of where the greatest proportions of *Salmonell*a

were detected by the researchers. Other studies have reported similar results, in particular greater growth or survival in stem scar tissue as opposed to edible tomato surface (Beuchat and Mann, 2008; Das *et al.*, 2006; Guo *et al.*, 2002; Wei *et al.*, 1995).

Although Beuchat and Brackett (1991) found *L. monocytogenes* to increase on inoculated tomatoes at 21°C for the first two days, the authors suggested that tomatoes were not a good growth substrate for the pathogen compared with low acid produce. Nonetheless, it was discovered by Iturriaga *et al.* (2003, 2007) using scanning electron microscopy that 0.3% of *Salmonella* Montevideo cells were able to adhere rapidly and irreversibly to the surfaces of tomatoes, with the authors attributing such attachment to surface waxiness. Due to the discrepancy among findings, limited understanding of microbe-plant interactions, and restricted scope of this study, we can only hypothesize that differences in contamination be somewhat attributed to differences in plant surfaces.

Low acidity

Cantaloupes are categorized as a low acid or non-acid food, with pH > 5.3 (Banwart, 1989). Specifically, the pH of cantaloupes range from 6.2 to 6.9 (Golden *et al.*, 1993), whereas the pH of naturally acidic produce, such as peppers and tomatoes, range from 4.65 to 5.45 (UW Food Safety & Health) and from 4.0 to 4.5, respectively (Jones, 2007). The lower acidity of cantaloupes may be a contributing factor to its greater surface contamination, as most microorganisms, including foodborne pathogens, grow optimally around pH 7.0 (Banwart, 1989). Specifically, *E. coli* grow optimally from pH 6.0 to 8.0 (Banwart, 1989), *Enterococcus* species at pH 7.5 (Van den Berghe *et al.*, 2006), and total coliforms from pH 6.0 to 7.0 (Adhikari

et al., 2007). Thus, the growth of microorganisms may be better supported on cantaloupes as opposed to on peppers and tomatoes.

Studies have generally found that fruits with pH below 4.0 are not good substrates for bacterial growth (Banwart, 1989; Conner and Kotrola, 1995; Parish and Higgins, 1989). Meanwhile, studies have reported pathogenic growth in and on low acid fruits. For instance, Pao *et al.* (1998) reported growth of *Salmonella*, *E. coli* 0157:H7, *L. monocytogenes,* and *Staphylococcus aureus* on peeled Hamlin orange, which has surface pH 6.0 to 6.5. Furthermore, Penteado and Leitao (2004) reported that pulps of low acid fruits, such as melons, watermelons, and papaya, were good substrates for growth of *L. monocytogenes.*

Nevertheless, the extent to which pH of most produce inhibits pathogen growth may not be significant. Many of the studies mentioned in the previous section discussing produce surfaces did observe rapid and/or prolific pathogen multiplication in jalapeños or tomatoes that were sliced, chopped, or blended, thus directly exposing pathogens to acidic environments (Beuchat and Mann, 2008; Castro-Rosas *et al.*, 2011; Ma *et al.*, 2010; Zhuang *et al.*, 1995). Specifically, Wei *et al.* (1995) found that the low pH of tomatoes did not inhibit surface growth of *S.* Montevideo. Moreover, enteric pathogens and microorganisms have been detected in jalapeño sauces from restaurants (Adachi *et al.*, 2002) and street vendors (Cerna-Cortes *et al.*, 2009; Estrada-Garcia *et al.*, 2002).

Direct soil contact

Another factor that may be attributed to the differences in contamination among produce types is growing method. Cantaloupes are grown in direct contact with soil, whereas jalapeños and tomatoes are grown suspended on stakes. Such extensive soil contact during development

may have attributed to the greater contamination of cantaloupes in our study, in particular to the higher microbial contamination of cantaloupes at pre-harvest. Studies have found ground-growing crops to have greater contamination compared with crops grown staked; for instance, El Hamouri *et al.* (1996) reported cucumbers to have higher fecal contamination compared with tomatoes, as a result of soil contact. Similarly, Melloul *et al.* (2001) reported that lettuce and parsley, which are ground-growers, were more contaminated with *Salmonella* than were tomatoes and pimento grown suspended. In regards to cantaloupes, the region of the rind that contacts the ground during development, known as the ground spot, is usually thinner and less matured than non-ground spots and are more vulnerable to microbial growth (Castillo *et al.*, 2009). Moreover, laboratory analyses have found cantaloupe ground spots to have significantly higher microbial concentrations than non-ground spots (National Cantaloupe Guidance, 2013).

Growing cantaloupes on the ground may increase the crop's risk of contact with water during surface drip irrigation, when water is applied to soil surfaces. On the other hand, because tomatoes and jalapeños are grown on stakes, the crops generally do not have opportunities to come into contact with water that is surface drip irrigated. However, across all crop types, this study found no positive relationships between pre-harvest produce and associated irrigation water in terms of microbial contamination. Although a slight negative correlation in *E. coli* concentrations between cantaloupes and associated water was detected, this finding was likely coincidental. The lack of positive relationships in microbial contamination between cantaloupes and associated irrigation water may be attributed to the slow outflow rate of water from the irrigation hose during drip irrigation. Such techniques likely result in minimal to no contact of ground-growing crops with water. In summary, it is unlikely that contaminated irrigation water contributed to the greater contamination of cantaloupe surfaces.

Excessive handling

Due to the greater surface area and weight of cantaloupes, the melons may be handled more excessively by farmworkers during harvest, distribution, and packing compared with smaller crops. Excessive handling of cantaloupes provides more opportunities for cross-contamination, and farmworkers infected with pathogens have caused outbreaks involving produce such as green onions, strawberries, raspberries, tomatoes, leaf lettuce, basil, and parsley (Bihn and Gravani, 2006; Gravani, 2009; Michaels and Todd, 2006).

In addition, excessive handling may also make cantaloupes more prone to damages, and studies have associated wounded fruit with a higher risk for contamination (Dingman, 2000; Fatemi *et al.*, 2006; Wells and Butterfield, 1997). Specifically, studies have found that cuts, bruises, or punctures make produce more susceptible to microbial attachment and growth (Burnett *et al.*, 2000; Kenney *et al.*, 2001). In particular, *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* have been found to attach frequently to cracked or injured plant surfaces (Burnett *et al.*, 2000; Kenney *et al.*, 2001; Seo and Frank, 1999; Takeuchi and Frank, 2001; Takeuchi *et al.*, 2000). Multiple studies also have demonstrated the growth of *S*. Poona on wounded cantaloupe rinds (Beuchat and Scouten, 2004; Richards and Beuchat, 2004, 2005b).

Implications of greater cantaloupe contamination

Greater fecal contamination of cantaloupe surfaces may indicate greater risk for pathogen contamination. Furthermore, as pathogens on cantaloupe rinds can be transferred to the internal flesh, there may be greater risk of pathogen consumption. Studies have observed transfer of *Salmonella* and *L. monocytogenes* from rind to flesh by either direct contact or cutting (Ukuku and Sapers, 2001; Ukuku and Fett, 2002a). Outbreaks have resulted from the consumption of

cantaloupes of which *Salmonella* on the rind had been internalized (Beuchat, 1996; CDC, 1991, 1996; CDR, 1991; Mohle-Boetani *et al.*, 1999).

Pathogen internalization can also occur in whole, uncut cantaloupes via the stem scar or ground spot (Castillo *et al.*, 2004; Richards and Beuchat, 2004; Ukuku and Fett, 2002b). Injuries of the rind may also facilitate internalization. Studies have found cracked or wounded cantaloupe netting to promote *Salmonella* infiltration into the flesh (Annous *et al.*, 2004); in particular, Richards and Beuchat (2005a) found that *S*. Poona inoculated on wounded cantaloupe rinds at a depth of 4 mm could migrate to a depth of 3 to 4 cm.

Once transferred to the flesh, pathogens can survive and multiply. Golden *et al.* (1993) found *Salmonella* to grow rapidly in cantaloupe cubes for 24 hours at 23°C. Escartin *et al.* (1989) found *Salmonella* and *Shigella* to populate on the cut surface of cantaloupes at 23°C. Similarly, Del-Rosario and Beuchat (1995) found *E. coli* O157:H7 to multiply in cantaloupe cubes at 25°C.

Tomato associated irrigation water has greater contamination

Tomato associated irrigation water was found to have significantly higher *E. coli* concentrations compared with jalapeño and cantaloupe associated water. Furthermore, tomato associated water was significantly more likely to be positive for *E. coli* than were jalapeño and cantaloupe associated water as well as more likely to be positive for somatic coliphage as was cantaloupe associated water. No relationships in microbial contamination between tomato associated irrigation water and pre-harvest tomatoes were detected.

Microbial concentrations in irrigation water samples were compared with the 2012 Recreational Water Quality Criteria of the Environmental Protection Agency, which

recommends that the geometric mean *Enterococcus* and *E. coli* concentrations not exceed 30 CFU/100 ml and 100 CFU/100 ml in any 30-day period, respectively (EPA, 2012). The EPA states that under such criteria, there is an estimated illness rate of 32 per 1,000 primary contact recreators. The geometric mean *Enterococcus* and *E. coli* concentrations of our samples for each farm never exceeded 10 CFU/100 ml and 0.4 CFU/100 ml in any 30-day interval, respectively. Our samples also complied with the designated statistical threshold values (110 CFU/100 ml for *Enterococcus* and 320 CFU/100 ml for *E. coli*), of which there should not be greater than a 10% excursion frequency in the same 30-day period. Thus, the differences in contamination among produce associated water are trivial, as overall water quality used by our farms was satisfactory.

Proposed mechanisms for greater contamination of tomato associated irrigation water

The farms in our study used well water as the source of irrigation. The differences in microbial concentrations and prevalence among produce associated water can be attributed to the different wells used by the farms. We collected water samples from one exclusively tomato-growing farm, two exclusively jalapeño-growing farms, and five exclusively cantaloupe-growing farms, with three farms growing both tomatoes and jalapeños. Each of these three farms used the same well to irrigate both crop types, and as expected, tomato and jalapeño associated water samples collected from each of these farms had similar microbial concentrations (data not shown). Furthermore, when statistical comparisons were redone using only samples from the one exclusively tomato-growing farm to represent tomato associated water, the same results were found (data not shown). This suggests that the greater microbial contamination of tomato associated water was likely a result of greater contamination of the well water used by the one exclusively tomato-growing farm.

Despite undergoing natural filtration, water extracted from wells may not be free from pathogens or microorganisms. Recently, it was reported that 8 to 31% of groundwater in the U.S. may be contaminated with viruses as a result of faulty septic systems or contamination with oxidation ponds, rivers, or lakes (Abbaszadegan *et al.*, 2003; Borchardt *et al.*, 2003). Furthermore, pathogens may infiltrate into wells through leaks or damages in the structure, as well as due to poor design or unsanitary practices (CDC, 2009).

A study done in Texan farms examined water samples collected from various sources used for irrigation and found that well water samples had significantly higher *E. coli* concentrations (mean of 0.70 ± 0.3 $\log_{10}$ CFU/ml) compared with samples from the Rio Grande River, cement and dirt irrigation canals, and furrows (Duffy *et al.*, 2005). Furthermore, at a limit of detection of 1 CFU/ml, 100% of well water samples were positive for *E. coli*, while only 75% of furrow samples, 50% of reservoir and dirt canal samples, 30% of river samples, and 6% of cement canal samples were positive. Such findings suggest that well water may not necessarily be free from fecal contamination or have higher quality than other irrigation water sources.

However, because of groundwater filtration, well water is generally less contaminated with microorganisms compared with open water bodies such as rivers or canals. For instance, Castillo *et al.* (2004) did not detect *E. coli* or *Salmonella* in water samples collected from farms using wells for irrigation. On the other hand, 93.3% and 67% of water samples collected from a farm using a canal for irrigation were contaminated with *E. coli* and *Salmonella*, respectively. Among farms that used river water from the Rio Grande for irrigation, the authors also reported many samples positive for *E. coli*.

Implications of greater irrigation water contamination

Numerous studies have found that contaminated water used for irrigation can result in subsequent crop contamination. For instance, Erickson *et al.* (2010) found a positive correlation between *E. coli* O157:H7 concentrations in irrigation water and occurrence on spinach. Patel and Darlington (2010) detected *Salmonella* on spinach plants (10^4 CFU/plant) when plants were irrigated with a high concentration of *Salmonella* (10^6 CFU/ml) but not when irrigated with a low concentration (10^3 CFU/ml). Mootian *et al.* (2009) found that 30% of mature lettuce plants became contaminated with *E. coli* O157:H7 after 15 days of irrigation with water harboring the pathogen in low concentrations of 10^1 or 10^2 CFU/ml.

However, different methods of irrigation affect the likelihood and extent of associated crop contamination. Common irrigation systems include surface, sprinkler, and drip irrigation. Surface irrigation consists of water flowing over soil surfaces; either the entire field is flooded or just between rows of crops (furrow irrigation). Sprinkler irrigation sprays water through nozzles, such that produce surfaces are often contacted. Drip irrigation sends water directly onto the soil surface through hoses (surface drip) or directly to crop roots belowground (subsurface drip). Drip irrigation reduces opportunities for water to contact edible produce surfaces, and thus, the likelihood of contamination of produce grown on stakes (Pescod, 1992).

Typically, water-crop contact is greatest when using spray or sprinkler irrigation, followed by flood irrigation, furrow irrigation, surface drip irrigation, and subsurface drip irrigation. Studies suggest a positive relationship between water-crop contact and crop contamination. Solomon *et al.* (2002) found that nearly 91% of lettuce spray irrigated with *E. coli* O157:H7 inoculated water became contaminated, as opposed to less than 19% of lettuce surface irrigated. Moreover, Song *et al.* (2006) found a 99.9% and 99% decrease in *E. coli* and

coliphage PRD-1 concentrations, respectively, on lettuce when using subsurface drip rather than flood irrigation. Other researchers have determined transfer rates of coliphage PRD-1 to lettuce to be 4.4% for spray, 0.02% for furrow, and 0.00039% for drip irrigation (Choi *et al.*, 2004; Stine *et al.*, 2005a; Stine *et al.*, 2005b).

The lack of relationships found in our study regarding microbial contamination between produce and associated surface drip irrigation water further support that water-crop contact is a major factor in crop contamination. In particular, tomatoes and jalapeños, which were grown on stakes, were unlikely to come into contact with water that was surface drip irrigated. Furthermore, the lack of positive relationships in microbial contamination between cantaloupes and associated irrigation water in our study also suggests that when surface drip irrigation is used, water-crop contact is minimal even for ground-growing crops. However, it is possible that microbial concentrations in our irrigation water were too low to have any substantial effects on cantaloupe contamination. Ultimately, our findings suggest that water that is surface drip irrigated and that has similar quality as our samples likely poses little risk for contamination of ground-growing crops.

Recommendations for farms

Not only did cantaloupes have greater microbial contamination compared with the other produce types at pre-harvest, but the degree of contamination experienced at pre-harvest did not change during later production stages. Such findings suggest that interventions aimed to reduce produce contamination on the farm should target crops during the pre-harvest stage. Thus, we recommend jalapeño, tomato, and especially cantaloupe growers to implement practices that reduce risk of contamination during crop growth. Recommendations for cantaloupe growers include growing cantaloupes on barriers in the field to prevent direct ground contact (National

Cantaloupe Guidance, 2013). Recommendations for jalapeño, tomato, and cantaloupe growers include making sure that fertilizer applied to crop fields be adequately treated to inactivate pathogens (FDA, 2014). Lastly, wild and domestic animals should not have access to fields (FDA, 2014). Overall, we recommend that all farms, regardless of crop type being grown, maintain good agricultural practices (GAPs), such as proper farmworker hygiene and sanitation and the use of clean tools and equipment on the farm (FDA, 2014).

Overall, the quality of irrigation water used by our farms was satisfactory, with microbial concentrations far below EPA standards. Because our study found no relationships between produce and irrigation water in regards to contamination, we recommend continuing the use of clean well water for irrigation as well as use of drip irrigation. Regular testing of well water is advised, as well as inspections of well conditions, repairing as needed.

Strengths and limitations

Strengths in this study include sampling of a wide variety of farms within a large agricultural region, as well as the implementation of a random sampling scheme. Limitations of this study include small sample sizes, in particular of produce samples at the packing shed. Such small sample sizes may not have been well representative of the study population, and in some cases rendered incomplete statistical analyses. Another disadvantage involved our study design, in that we sampled from some farms that grew more than one type of our study crop and some that grew only one crop type. Thus, our findings in regards to contamination among produce types could also be attributed to differences, or lack of differences, in agricultural practices or environmental conditions on the farms. In addition, our data did not always meet the Kruskal-Wallis test assumption that groups under comparison have similarly shaped distributions

(Figures 1, 2) which may have affected the accuracy of our results (Fagerland and Sandvik, 2009). Lastly, we used indicator organisms as surrogates for enteric pathogens, the two of which may not be strongly correlated (Horman *et al.*, 2004; Lemarchand and Lebaron, 2003; Lipp *et al.*, 2001; Morinigo *et al.*, 1990; Payment *et al.*, 2000).

Future research

Our study may not have been able to detect significant differences in mean microbial concentrations or prevalence due to inadequate sample size. However, based on our power analyses, future studies with appropriate sample sizes may have the power to detect such differences. Specifically, the appropriate sample sizes to detect mean differences in microbial concentrations among cantaloupes, tomatoes, and jalapeños are for each indicator: fecal coliforms (37 to 122), *E. coli* (6 to 45), *Enterococcus* (9 to 800), and somatic coliphage (91 to 33,241) (Table 12). The appropriate sample sizes to detect differences in microbial prevalence are: fecal coliforms (73 to 283), *E. coli* (11 to 19,460), *Enterococcus* (16 to 915), and somatic coliphage (26 to 1509) (Table 13).

Future studies may compare the attachment, survival, growth, or detachment of pathogens among cantaloupes, jalapeños, tomatoes, and other crops to understand what specific plant characteristics promote or inhibit pathogens. Studies may also examine the correlation between indicator and pathogen contamination among crop types. Most importantly, future research should focus on understanding what the dominant risk factors for contamination are at the pre-harvest stage and how they may vary among different crops, in order to develop produce-specific interventions to reduce or prevent contamination on the farm.

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Table 1. Calculations performed for seven types of assay values.

Type	CFU/assay values represented					CFU/ml calculation across replicate assays	
	0	1-24	25-250	>250	TNTC	Numerator	Denominator
1	✓	X	X	X	X	0.5	Maximum EV assayed
2		✓	X	X	X	ΣCFU values from assays with largest EV	Σ corresponding EV
3			✓			ΣCFU values between 25-250	Σ corresponding EV
4	X	X	X	✓		ΣCFU values from assays with smallest EV	Σ corresponding EV
5		✓	X	✓		ΣCFU values from assays with largest EV	Σ corresponding EV
6	X	X	X	X	✓	500	Minimum EV assayed
7	✓	X	X	X	✓	500	Minimum EV assayed

CFU = Colony Forming Unit

EV = Effective Volume

TNTC = Too Numerous To Count

✓= Must include

X = Must not include

Blank = Does not matter

Table 2. Limits of detection (LOD) and quantification (LOQ) for assays[#].

Indicator	Produce type	Produce				Irrigation Water	
		log_{10} CFU or MPN / fruit		log_{10} CFU or MPN / ml		log_{10} CFU or MPN / 100 ml	
		Lower LOD	Upper LOQ	Lower LOD	Upper LOQ	Lower LOD	Upper LOQ
Fecal coliforms & *E. coli*	Cantaloupe	0.6990	5.7959	-1.6990	3.3979	-0.3979	3.3979
	Jalapeño	-0.1487	4.9508				
	Tomato	-0.2518	4.8416				
Enterococcus	Cantaloupe	1.3979	6.7959	-1.0000	4.3979	-0.3979	3.3979
	Jalapeño	0.5527	5.9508				
	Tomato	0.4440	5.8416				
Somatic coliphage	Cantaloupe	0.3979	3.7818	-2.0000	1.3838	0.0000	3.3838
	Jalapeño	-0.4437	2.9367				
	Tomato	-0.5528	2.8275				

[#]LOD and LOQ values for produce measured in ml and for irrigation water are the same across produce types for each indicator.

Table 3. Comparisons of microbial concentrations among cantaloupes, jalapeños, and tomatoes combined from all production stages and associated irrigation water.

		Kruskal-Wallis[#]		Steel-Dwass[¥]					
				Cantaloupe		Jalapeño		Tomato	
Sample type	Indicator	Chi-square	p-value	Geometric mean (95% CI)	n[ψ]	Geometric mean (95% CI)	n[ψ]	Geometric mean (95% CI)	n[ψ]
Produce (log_{10} CFU or MPN/fruit)	Fecal coliforms	58.4126	<0.0001*	6.49 (6.27, 6.71)^{A}	106	3.88 (3.24, 4.52)^{B}	61	4.59 (4.17, 5.00)^{B}	83
	E. coli	124.4319	<0.0001*	2.83 (2.44, 3.21)^{A}	106	0.27 (-0.02, 0.56)^{B}	64	0.19 (-0.06, 0.44)^{C}	84
	Enterococcus	135.1407	<0.0001*	7.20 (6.90, 7.49)^{A}	106	3.58 (3.10, 4.05)^{B}	64	3.50 (3.17, 3.83)^{B}	84
	Somatic coliphage	99.9704	<0.0001*	3.64 (3.35, 3.93)^{A}	79	1.22 (0.77, 1.67)^{B}	46	0.91 (0.61, 1.22)^{B}	66
Produce (log_{10} CFU or MPN/ml)	Fecal coliforms	28.8826	<0.0001*	4.09 (3.87, 4.32)^{A}	106	2.33 (1.69, 2.97)^{C}	61	3.14 (2.72, 3.56)^{B}	83
	E. coli	54.9995	<0.0001*	0.43 (0.04, 0.82)^{A}	106	-1.28 (-1.57, -1.00)^{B}	64	-1.25 (-1.50, -1.00)^{B}	84
	Enterococcus	104.1197	<0.0001*	4.80 (4.50, 5.10)^{A}	106	2.02 (1.55, 2.49)^{B}	64	2.06 (1.73, 2.39)^{B}	84
	Somatic coliphage	64.5045	<0.0001*	1.24 (0.96, 1.53)^{A}	79	-0.33 (-0.78, 0.12)^{B}	46	-0.53 (-0.84, -0.22)^{B}	66
Irrigation Water (log_{10} CFU or MPN/100 ml)	Fecal coliforms	0.4364	0.8039	1.74 (1.25, 2.24)	38	1.54 (0.79, 2.28)	14	1.57 (1.22, 1.93)	21
	E. coli	10.1573	0.0062*	-0.17 (-0.48, 0.13)^{B}	38	-0.50 (-0.78, -0.22)^{B}	15	0.32 (-0.05, 0.69)^{A}	23
	Enterococcus	1.9257	0.3818	0.53 (0.04, 1.02)	38	0.60 (0.00, 1.20)	14	0.46 (0.16, 0.77)	23
	Somatic coliphage	3.0470	0.2179	0.75 (0.16, 1.34)	30	1.36 (-0.15, 2.88)	7	1.49 (0.52, 2.46)	10

[#]Significant chi-square is indicated by p-value with asterisk, and indicates one or more significant differences in mean rank indicator concentrations between or among produce types (α=0.05).

[¥]Produce types with different superscript letters have significantly different microbial concentrations (α=0.05), with letters representing highest to lowest microbial concentrations in alphabetical order. Lack of a letter set indicates no significant differences across produce types.

[ψ]Column includes sample sizes at each production stage.

Table 4. Comparisons of microbial concentrations measured in units of log_{10} CFU or MPN/fruit among cantaloupes, jalapeños, and tomatoes at each production stage.

		Kruskal-Wallis[#]		Steel-Dwass[¥]					
Indicator	Production Stage	Chi-square	p-value	Cantaloupe Geometric mean (95% CI)	n[ψ]	Jalapeño Geometric mean (95% CI)	n[ψ]	Tomato Geometric mean (95% CI)	n[ψ]
Fecal coliforms (log_{10} CFU/fruit)	Pre-Harvest	18.1374	0.0001*	6.51 (6.17, 6.84)^{A}	37	4.11 (2.98, 5.24)^{B}	20	4.80 (4.07, 5.53)^{B}	25
	Harvest	12.0030	0.0025*	6.18 (5.78, 6.59)^{A}	38	4.00 (2.68, 5.33)^{B}	20	4.89 (4.15, 5.64)^{B}	25
	Distribution	20.4325	<0.0001*	6.75 (6.30, 7.19)^{A}	22	3.55 (2.45, 4.65)^{B}	20	4.56 (3.81, 5.31)^{B}	25
	Packing Shed	7.3606	0.0252*	7.09 (5.90, 8.28)^{A}	9	3.55 (N/A, N/A)^{AB}	1	3.04 (0.90, 5.17)^{B}	8
E. coli (log_{10} CFU/fruit)	Pre-Harvest	39.3706	<0.0001*	2.34 (1.72, 2.96)^{A}	37	0.19 (-0.37, 0.75)^{B}	21	0.11 (-0.35, 0.57)^{C}	26
	Harvest	40.9700	<0.0001*	2.23 (1.61, 2.85)^{A}	38	0.12 (-0.36, 0.60)^{B}	21	0.07 (-0.42, 0.56)^{C}	25
	Distribution	42.5503	<0.0001*	3.60 (2.92, 4.28)^{A}	22	0.29 (-0.19, 0.77)^{B}	20	0.10 (-0.34, 0.53)^{C}	25
	Packing Shed	12.8004	0.0017*	5.47 (4.28, 6.66)^{A}	9	2.41 (-12.10, 16.92)^{AB}	2	1.14 (0.13, 2.14)^{B}	8
Enterococcus (log_{10} CFU/fruit)	Pre-Harvest	47.7797	<0.0001*	7.16 (6.65, 7.67)^{A}	37	3.31 (2.50, 4.11)^{B}	21	3.23 (2.58, 3.88)^{B}	26
	Harvest	42.2423	<0.0001*	7.11 (6.63, 7.60)^{A}	38	3.65 (2.76, 4.54)^{B}	21	3.78 (3.15, 4.41)^{B}	25
	Distribution	28.7414	<0.0001*	7.22 (6.41, 8.04)^{A}	22	3.69 (2.76, 4.61)^{B}	20	3.76 (3.11, 4.41)^{B}	25
	Packing Shed	13.8749	0.0010*	7.64 (6.61, 8.67)^{A}	9	4.46 (-19.78, 28.71)^{AB}	2	2.73 (2.16, 3.30)^{B}	8
Somatic coliphage (log_{10} MPN/fruit)	Pre-Harvest	42.2855	<0.0001*	3.81 (3.42, 4.20)^{A}	29	1.78 (1.07, 2.49)^{B}	15	0.68 (0.08, 1.28)^{C}	20
	Harvest	29.8802	<0.0001*	3.45 (2.88, 4.03)^{A}	30	0.76 (-0.07, 1.59)^{B}	15	0.91 (0.28, 1.54)^{B}	19
	Distribution	18.2672	0.0001*	3.51 (2.68, 4.35)^{A}	14	1.10 (0.19, 2.02)^{B}	14	0.81 (0.27, 1.35)^{B}	19
	Packing Shed	11.2324	0.0036*	4.08 (N/A, N/A)^{A}	6	1.25 (-24.08, 26.57)^{B}	2	1.76 (0.78, 2.74)^{B}	8

[#]Significant chi-square is indicated by p-value with asterisk, and indicates one or more significant differences in mean rank indicator concentrations between or among produce types (α=0.05).

[¥]Produce types with different superscript letters have significantly different microbial concentrations (α=0.05), with letters representing highest to lowest microbial concentrations in alphabetical order. Lack of a letter set indicates no significant differences across produce types.

[ψ]Column includes sample sizes at each production stage.

Table 5. Comparisons of microbial concentrations measured in units of log_{10} CFU or MPN/ml among cantaloupes, jalapeños, and tomatoes at each production stage.

		Kruskal-Wallis[#]		Steel-Dwass[¥]					
				Cantaloupe		Jalapeño		Tomato	
Indicator	Production Stage	Chi-square	p-value	Geometric mean (95% CI)	n[ψ]	Geometric mean (95% CI)	n[ψ]	Geometric mean (95% CI)	n[ψ]
Fecal coliforms (log_{10} CFU/ml)	Pre-Harvest	8.0371	0.0180*	4.11 (3.77, 4.44)^{A}	37	2.56 (1.42, 3.69)^{B}	20	3.35 (2.63, 4.08)^{AB}	25
	Harvest	4.3375	0.1143	3.79 (3.38, 4.19)	38	2.45 (1.13, 3.78)	20	3.45 (2.70, 4.19)	25
	Distribution	13.8395	0.0010*	4.35 (3.91, 4.80)^{A}	22	2.00 (0.90, 3.09)^{B}	20	3.12 (2.37, 3.87)^{B}	25
	Packing Shed	7.3606	0.0252*	4.69 (3.50, 5.88)^{A}	9	2.00 (N/A, N/A)^{AB}	1	1.59 (-0.54, 3.72)^{B}	8
E. coli (log_{10} CFU/ml)	Pre-Harvest	14.1565	0.0008*	-0.06 (-0.68, 0.56)^{A}	37	-1.36 (-1.92, -0.81)^{B}	21	-1.34 (-1.80, -0.88)^{B}	26
	Harvest	14.8049	0.0006*	-0.17 (-0.79, 0.45)^{A}	38	-1.43 (-1.91, -0.95)^{B}	21	-1.37 (-1.86, -0.88)^{B}	25
	Distribution	28.9172	<0.0001*	1.20 (0.52, 1.89)^{A}	22	-1.27 (-1.75, -0.78)^{B}	20	-1.35 (-1.78, -0.91)^{B}	25
	Packing Shed	11.9620	0.0025*	3.08 (1.88, 4.27)^{A}	9	0.86 (-13.65, 15.37)^{AB}	2	-0.31 (-1.31, 0.69)^{B}	8
Enterococcus (log_{10} CFU/ml)	Pre-Harvest	41.5559	<0.0001*	4.76 (4.25, 5.27)^{A}	37	1.75 (0.95, 2.56)^{B}	21	1.79 (1.14, 2.43)^{B}	26
	Harvest	32.2138	<0.0001*	4.71 (4.23, 5.20)^{A}	38	2.10 (1.21, 2.99)^{B}	21	2.33 (1.70, 2.96)^{B}	25
	Distribution	20.7001	<0.0001*	4.83 (4.02, 5.64)^{A}	22	2.14 (1.21, 3.06)^{B}	20	2.32 (1.66, 2.97)^{B}	25
	Packing Shed	11.9622	0.0025*	5.24 (4.21, 6.27)^{A}	9	2.91 (-21.34, 27.15)^{AB}	2	1.29 (0.72, 1.86)^{B}	8
Somatic coliphage (log_{10} MPN/ml)	Pre-Harvest	24.0366	<0.0001*	1.41 (1.02, 1.80)^{A}	29	0.23 (-0.49, 0.94)^{B}	15	-0.77 (-1.36, -0.17)$_{B}$	20
	Harvest	23.4746	<0.0001*	1.05 (0.48, 1.63)^{A}	30	-0.79 (-1.62, 0.04)^{B}	15	-0.53 (-1.16, 0.09)$_{B}$	19
	Distribution	15.0143	0.0005*	1.12 (0.28, 1.95)^{A}	14	-0.45 (-1.37, 0.47)^{B}	14	-0.64 (-1.18, -0.09)$_{B}$	19
	Packing Shed	4.3352	0.1145	1.69 (N/A, N/A)	6	-0.31 (-25.63, 25.02)	2	0.31 (-0.67, 1.30)	8

[#]Significant chi-square is indicated by p-value with asterisk, and indicates one or more significant differences in mean rank indicator concentrations between or among produce types (α=0.05).

[¥]Produce types with different superscript letters have significantly different microbial concentrations (α=0.05), with letters representing highest to lowest microbial concentrations in alphabetical order. Lack of a letter set indicates no significant differences across produce types.

[ψ]Column includes sample sizes at each production stage.

Table 6. Comparisons of microbial prevalence among cantaloupes, jalapeños, and tomatoes combined from all production stages.

Indicator	Outcome[ψ]		Predictor[ψ]		Odds Ratio[#] (95% CI)	p-value[#]
	Produce type	Prevalence[¥]	Produce type	Prevalence[¥]		
Fecal coliforms	Cantaloupe[a]	106/106 (100%)	Jalapeño[a]	56/61 (92%)	.	.
	Cantaloupe	106/106 (100%)	Tomato	81/83 (98%)	.	.
	Tomato	81/83 98%)	Jalapeño	56/61 (92%)	.	.
E. coli	Cantaloupe	43/106 (41%)	Jalapeño	10/64 (16%)	3.6857 (1.7479, 8.3978)	0.0004*
	Cantaloupe	43/106 (41%)	Tomato	16/84 (19%)	2.9008 (1.5103, 5.7868)	0.0012*
	Tomato	16/84 (19%)	Jalapeño	10/64 (16%)	1.2706 (0.5401, 3.1108)	0.5862
Enterococcus	Cantaloupe	105/106 (99%)	Jalapeño	45/64 (70%)	44.3333 (8.7857, 808.1939)	<0.0001*
	Cantaloupe	105/106 (99%)	Tomato	65/84 (77%)	30.6923 (6.1412, 557.7007)	<0.0001*
	Tomato	65/84 (77%)	Jalapeño	45/64 (70%)	1.4444 (0.6869, 3.04427)	0.3308
Somatic coliphage	Cantaloupe	70/79 (89%)	Jalapeño	36/46 (78%)	2.1605 (0.8023, 5.9052)	0.1263
	Cantaloupe	70/79 (89%)	Tomato	53/66 (80%)	1.9078 (0.7659, 4.9413)	0.1657
	Tomato	53/66 (80%)	Jalapeño	36/46 (78%)	1.1325 (0.4396, 2.8546)	0.7928

[¥]Prevalence is shown as the number of positive samples / total number of samples tested (percentage of positive samples).

[#]Significant odds ratios for each produce pair are indicated by p-values with an asterisk (α=0.0167; Bonferroni corrected). Dots for a produce pair indicate inability to calculate odds ratio due to inadequate sample size or 100% prevalence, in which instances, Fisher's 2x2 Test was conducted, with significant pairwise differences labeled by a letter superscript (α=0.0167; Bonferroni corrected).

[a]Significant difference in fecal coliform prevalence between cantaloupes and jalapeños (p=0.0058*).

[ψ]Odds ratios are interpreted with outcome group relative to predictor group (i.e., cantaloupes were 3.6857 times more likely to be positive for *E. coli* compared with jalapeños).

Table 7. Comparisons of microbial prevalence among cantaloupes, jalapeños, and tomatoes at each production stage.

Indicator	Production Stage	p-value[¥]	Prevalence[#] Cantaloupe	Jalapeño	Tomato
Fecal coliforms	Pre-Harvest	0.0925	37/37 (100%)	18/20 (90%)	24/25 (96%)
	Harvest	0.2410	38/38 (100%)	19/20 (95%)	25/25 (100%)
	Distribution	0.3868	22/22 (100%)	18/20 (90%)	24/25 (96%)
	Packing Shed	.	9/9 (100%)	1/1 (100%)	8/8 (100%)
E. coli	Pre-Harvest	0.0360*	15/37 (41%)	3/21 (14%)	4/26 (15%)
	Harvest	0.0315*	11/38 (29%)	1/21 (5%)	2/25 (8%)
	Distribution	0.3224	9/22 (41%)	4/20 (20%)	6/25 (24%)
	Packing Shed	0.1620	8/9 (89%)	2/2 (100%)	4/8 (50%)
Enterococcus	Pre-Harvest	0.0002*	37/37 (100%)[ab]	14/21 (67%)[a]	19/26 (73%)[b]
	Harvest	0.0032*	38/38 (100%)[c]	16/21 (76%)[c]	21/25 (84%)
	Distribution	0.0457*	21/22 (96%)	13/20 (65%)	20/25 (80%)
	Packing Shed	0.1331	9/9 (100%)	2/2 (100%)	5/8 (63%)
Somatic coliphage	Pre-Harvest	0.0383*	27/29 (93%)	15/15 (100%)	15/20 (75%)
	Harvest	0.4263	25/30 (83%)	10/15 (67%)	15/19 (79%)
	Distribution	0.7521	12/14 (86%)	10/14 (71%)	15/19 (79%)
	Packing Shed	0.1250	6/6 (100%)	1/2 (50%)	8/8 (100%)

[¥]Significant Fisher's two-sided (2x3) test is indicated by p-value with asterisk (α=0.05). Dot in place of p-value indicates inability to run Fisher's test due to 100% prevalence across all produce types.

[#]Prevalence is shown as the number of positive samples / total number of samples tested (percentage of positive samples).

[#]Produce pairs with the same letter superscript have a significant difference in prevalence detected by Fisher's 2x2 test (α=0.0167; Bonferroni corrected): [a]p=0.0004*; [b]p=0.0012*; [c]p=0.0041*

Table 8. Comparisons of microbial prevalence among produce associated irrigation water.

Indicator	Outcome[ψ]		Predictor[ψ]		Odds Ratio[#] (95% CI)	p-value[#]
	Produce type	Prevalence[¥]	Produce type	Prevalence[¥]		
Fecal coliforms	Jalapeño	12/14 (86%)	Cantaloupe	35/38 (92%)	.	.
	Tomato	21/21 (100%)	Jalapeño	12/14 (86%)	.	.
	Tomato	21/21 (100%)	Cantaloupe	35/38 (92%)	.	.
E. coli	Jalapeño	3/15 (20%)	Cantaloupe	12/38 (32%)	0.5417 (0.1087, 2.1017)	0.3887
	Tomato	16/23 (70%)	Jalapeño	3/15 (20%)	9.1429 (2.1462, 50.4989)	0.0022*
	Tomato	16/23 (70%)	Cantaloupe	12/38 (32%)	4.9524 (1.6702, 16.0244)	0.0036*
Enterococcus	Jalapeño	13/14 (93%)	Cantaloupe	29/38 (76%)	4.0344 (0.6537, 78.3151)	0.1468
	Tomato	21/23 (91%)	Jalapeño	13/14 (93%)	0.8077 (0.0355, 9.2675)	0.8657
	Tomato	21/23 (91%)	Cantaloupe	29/38 (76%)	3.2586 (0.7431, 22.8395)	0.1231
Somatic coliphage	Jalapeño	4/7 (57%)	Cantaloupe	9/30 (30%)	3.1111 (0.5755, 18.6993)	0.1846
	Tomato	8/10 (80%)	Jalapeño	4/7 (57%)	3.0000 (0.3565, 31.1035)	0.3105
	Tomato	8/10 (80%)	Cantaloupe	9/30 (30%)	9.3333 (1.8980, 70.6194)	0.0050*

[¥]Prevalence is shown as the number of positive samples / total number of samples tested (percentage of positive samples).

[#]Significant odds ratios for each produce pair are indicated by p-values with an asterisk (α=0.0167; Bonferroni corrected). Dots for a produce pair indicate inability to calculate odds ratio due to inadequate sample size or 100% prevalence, in which instances, Fisher's 2x2 Test was conducted, with no significant pairwise differences detected (α=0.0167; Bonferroni corrected).

[ψ]Odds ratios are interpreted with outcome group relative to predictor group (i.e., tomato associated water was 4.9524 times more likely to be positive for *E. coli* compared with cantaloupe associated water).

Table 9. Correlation of microbial concentrations between pre-harvest produce (log_{10} CFU or MPN/fruit) and associated irrigation water (log_{10} CFU or MPN/100 ml).

Produce type	Indicator	Rho[#]	p-value[¥]
Cantaloupe	Fecal coliforms	-0.1978	0.2406
	E. coli	-0.3714	0.0236*
	Enterococcus	-0.2137	0.2040
	Somatic coliphage	-0.0897	0.6434
Jalapeño	Fecal coliforms	0.3333	0.2442
	E. coli	0.5013	0.0570
	Enterococcus	0.0597	0.8393
	Somatic coliphage	0.1538	0.7419
Tomato	Fecal coliforms	-0.2338	0.3076
	E. coli	0.1325	0.5469
	Enterococcus	-0.2117	0.3321
	Somatic coliphage	-0.0862	0.8129

[#]Statistic ranges from -1 to 1, such that -1 represents a perfect negative correlation, 0 represents no correlation and 1 represents perfect correlation between microbial concentrations of water and produce.

[¥]Significant rho is indicated by p-value with asterisk (α=0.05).

Table 10. Association of microbial prevalence between pre-harvest produce and associated irrigation water.

Produce type	Indicator	Odds Ratio[#] (95% CI)	p-value[¥]
Cantaloupe	Fecal coliforms	.	.
	E. coli	1.0714 (0.2540, 4.3486)	0.9231
	Enterococcus	.	.
	Somatic coliphage	0.4211 (0.0153, 11.5247)	0.5623
Jalapeño	Fecal coliforms	.	.
	E. coli	.	.
	Enterococcus	.	.
	Somatic coliphage	.	.
Tomato	Fecal coliforms	.	.
	E. coli	.	.
	Enterococcus	.	.
	Somatic coliphage	3.0000 (0.0897, 106.9303)	0.5036

[#]Odd ratios are interpreted with outcome group (produce) relative to predictor group (water); i.e., tomatoes were 3 times more likely to be positive for coliphage compared with tomato associated water (not significant).

[¥]No significant associations in microbial prevalence between produce and irrigation water were detected (α=0.05). Dots indicate inability to calculate odds ratios due to inadequate sample size or 100% prevalence.

Table 11. Arithmetic means and standard deviations of microbial concentrations (log_{10} CFU or MPN/fruit) on cantaloupes, jalapeños, and tomatoes at each production stage[¥].

		Cantaloupe			Jalapeño			Tomato		
Indicator	Production Stage	n[Ψ]	Arithmetic Mean	Standard Deviation	n[Ψ]	Arithmetic Mean	Standard Deviation[#]	n[Ψ]	Arithmetic Mean	Standard Deviation
Fecal coliforms (log_{10} CFU/fruit)	Pre-Harvest	37	1.862902	0.13701	20	1.326549	0.57715	25	1.501696	0.37696
	Harvest	38	1.802973	0.19789	20	1.087854	1.10203	25	1.515663	0.39911
	Distribution	22	1.899583	0.14105	20	0.958506	0.94923	25	1.439924	0.40802
	Packing	9	1.937114	0.21843	1	1.267748	.	8	0.969382	0.93747
E. coli (log_{10} CFU/fruit)	Pre-Harvest	37	0.44201	0.99699	21	0.464776	1.04949	26	0.173064	0.99859
	Harvest	38	0.34057	1.04627	21	0.065241	1.1194	25	0.644734	0.4814
	Distribution	22	1.120752	0.71675	20	-0.20771	0.99566	25	-0.13659	1.38002
	Packing	9	1.660837	0.30459	2	0.752928	0.72807	8	0.035009	1.54157
Enterococcus (log_{10} CFU/fruit)	Pre-Harvest	37	1.94517	0.22589	21	1.083971	0.46606	26	1.143437	0.37835
	Harvest	38	1.936897	0.23953	21	1.18381	0.45864	25	1.262064	0.36276
	Distribution	22	1.943273	0.27797	20	1.179205	0.5076	25	1.243954	0.40496
	Packing	9	2.019907	0.17266	2	1.394331	0.64647	8	0.97928	0.23908
Somatic coliphage (log_{10} MPN/fruit)	Pre-Harvest	29	1.148797	0.96467	15	0.430904	0.76229	20	0.02509	0.74915
	Harvest	30	0.790623	1.42181	15	0.261153	0.82086	19	0.24522	0.63358
	Distribution	14	0.872386	1.3584	14	0.341815	0.83178	19	0.02411	0.68516
	Packing	6	1.406779	0	2	1.174859	.	8	0.340725	0.73607

[¥]Means and standard deviations used for power analysis of sample sizes required to detect mean differences between produce types (Table 12; see methods for details).

[#]Dots indicate inability to calculate standard deviation due to inadequate sample size.

[Ψ]Sample sizes are included in column n.

Table 12. Sample sizes required to detect existing differences in arithmetic mean microbial concentrations (log_{10} CFU or MPN/fruit) between produce types at each production stage.

Indicator	Production stage	Produce type		Sample size[#]
Fecal coliforms	Pre-harvest	Cantaloupe	Jalapeño	*
		Cantaloupe	Tomato	*
		Tomato	Jalapeño	122
	Harvest	Cantaloupe	Jalapeño	*
		Cantaloupe	Tomato	*
		Tomato	Jalapeño	59
	Distribution	Cantaloupe	Jalapeño	*
		Cantaloupe	Tomato	*
		Tomato	Jalapeño	37
	Packing Shed	Cantaloupe	Jalapeño	.
		Cantaloupe	Tomato	*
		Tomato	Jalapeño	.
E. coli	Pre-harvest	Cantaloupe	Jalapeño	*
		Cantaloupe	Tomato	*
		Tomato	Jalapeño	*
	Harvest	Cantaloupe	Jalapeño	*
		Cantaloupe	Tomato	*
		Tomato	Jalapeño	*
	Distribution	Cantaloupe	Jalapeño	*
		Cantaloupe	Tomato	*
		Tomato	Jalapeño	*
	Packing Shed	Cantaloupe	Jalapeño	6
		Cantaloupe	Tomato	*
		Tomato	Jalapeño	45
Enterococcus	Pre-harvest	Cantaloupe	Jalapeño	*
		Cantaloupe	Tomato	*
		Tomato	Jalapeño	800
	Harvest	Cantaloupe	Jalapeño	*
		Cantaloupe	Tomato	*
		Tomato	Jalapeño	439
	Distribution	Cantaloupe	Jalapeño	*
		Cantaloupe	Tomato	*
		Tomato	Jalapeño	790
	Packing Shed	Cantaloupe	Jalapeño	9
		Cantaloupe	Tomato	*
		Tomato	Jalapeño	22
Somatic coliphage	Pre-harvest	Cantaloupe	Jalapeño	*
		Cantaloupe	Tomato	*
		Tomato	Jalapeño	*
	Harvest	Cantaloupe	Jalapeño	*
		Cantaloupe	Tomato	*
		Tomato	Jalapeño	33241
	Distribution	Cantaloupe	Jalapeño	*
		Cantaloupe	Tomato	*
		Tomato	Jalapeño	91
	Packing Shed	Cantaloupe	Jalapeño	*
		Cantaloupe	Tomato	*
		Tomato	Jalapeño	.

[#]Sample size required for each produce type. Calculations based on 80% power, equal number of samples of both groups, and alpha level of 0.05. If pairwise comparison was found to be significant via Steel-Dwass (Table 4), a sample size was not calculated for this pair. Dots indicate inability to run analysis due to no standard deviation available for one produce type. See Table 11 for actual sample sizes, mean differences, and standard deviations used for analysis.

*Pairwise comparison was found significant via Steel-Dwass (α=0.05, Table 4), and therefore no sample size was calculated.

Table 13. Sample sizes required to detect existing differences in microbial prevalence between produce types at each production stage.

Indicator	Production stage	Produce type (# positive/total samples)		Sample size[#]
Fecal coliforms	Pre-harvest	Cantaloupe (37/37)	Jalapeno (18/20)	73
		Cantaloupe (37/37)	Tomato (24/25)	191
		Tomato (24/25)	Jalapeno (18/20)	283
	Harvest	Cantaloupe (38/38)	Jalapeno (19/20)	152
		Cantaloupe (38/38)	Tomato (25/25)	.
		Tomato (25/25)	Jalapeno (19/20)	152
	Distribution	Cantaloupe (22/22)	Jalapeno (18/20)	73
		Cantaloupe (22/22)	Tomato (24/25)	191
		Tomato (24/25)	Jalapeno (18/20)	283
	Packing Shed	Cantaloupe (9/9)	Jalapeno (1/1)	.
		Cantaloupe (9/9)	Tomato (8/8)	.
		Tomato (8/8)	Jalapeno (1/1)	.
E. coli	Pre-harvest	Cantaloupe (15/37)	Jalapeno (3/21)	42
		Cantaloupe (15/37)	Tomato (4/26)	46
		Tomato (4/26)	Jalapeno (3/21)	19460
	Harvest	Cantaloupe (11/38)	Jalapeno (1/21)	37
		Cantaloupe (11/38)	Tomato (2/25)	52
		Tomato (2/25)	Jalapeno (1/21)	1059
	Distribution	Cantaloupe (9/22)	Jalapeno (4/20)	74
		Cantaloupe (9/22)	Tomato (6/25)	118
		Tomato (6/25)	Jalapeno (4/20)	1682
	Packing Shed	Cantaloupe (8/9)	Jalapeno (2/2)	66
		Cantaloupe (8/9)	Tomato (4/8)	21
		Tomato (4/8)	Jalapeno (2/2)	11
Enterococcus	Pre-harvest	Cantaloupe (37/37)	Jalapeno (14/21)	*
		Cantaloupe (37/37)	Tomato (19/26)	*
		Tomato (19/26)	Jalapeno (14/21)	915
	Harvest	Cantaloupe (38/38)	Jalapeno (16/21)	*
		Cantaloupe (38/38)	Tomato (21/25)	44
		Tomato (21/25)	Jalapeno (16/21)	391
	Distribution	Cantaloupe (21/22)	Jalapeno (13/20)	24
		Cantaloupe (21/22)	Tomato (20/25)	64
		Tomato (20/25)	Jalapeno (13/20)	138
	Packing Shed	Cantaloupe (9/9)	Jalapeno (2/2)	.
		Cantaloupe (9/9)	Tomato (5/8)	16
		Tomato (5/8)	Jalapeno (2/2)	16
Somatic coliphage	Pre-harvest	Cantaloupe (27/29)	Jalapeno (15/15)	107
		Cantaloupe (27/29)	Tomato (15/20)	64
		Tomato (15/20)	Jalapeno (15/15)	26
	Harvest	Cantaloupe (25/30)	Jalapeno (10/15)	114
		Cantaloupe (25/30)	Tomato (15/19)	1509
		Tomato (15/19)	Jalapeno (10/15)	214
	Distribution	Cantaloupe (12/14)	Jalapeno (10/14)	117
		Cantaloupe (12/14)	Tomato (15/19)	461
		Tomato (15/19)	Jalapeno (10/14)	214
	Packing Shed	Cantaloupe (6/6)	Jalapeno (1/2)	11
		Cantaloupe (6/6)	Tomato (8/8)	.
		Tomato (8/8)	Jalapeno (1/2)	11

[#]Sample size required for each produce type. If pairwise comparison was found to be significant via Fisher's 2x2 test (Table 7), a sample size was not calculated for this pair. Calculations based on 80% power, equal number of samples of both groups, and alpha level of 0.05. Dots indicate inability to run analysis due to 100% of both produce types.

*Pairwise comparison was found significant via Fisher's 2x2 Test (α=0.0167; Bonferroni corrected; Table 7).

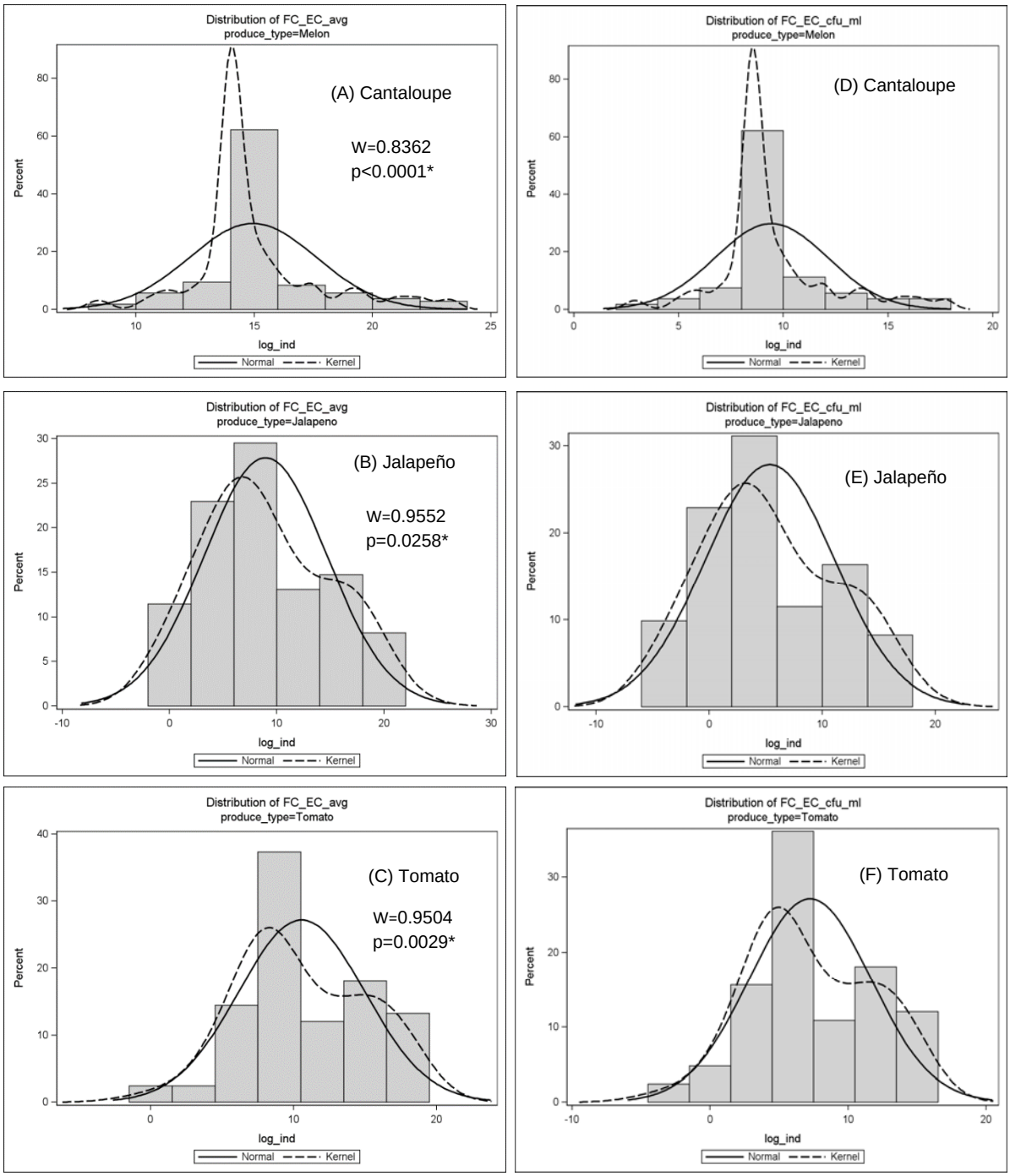

Figure 1A. Histograms illustrating fecal coliform distributions on each produce type and Shapiro-Wilk test results. Histograms A–C show concentrations (log_ind) in $\log_{10}$ CFU/fruit; D–F show concentrations in $\log_{10}$ CFU/ml. Dashed curve shows data distributions; solid curve shows normal distribution. Significant Shapiro-Wilk test statistic, W is indicated by p-value with asterisk (α=0.05) and indicates a non-normal distribution.

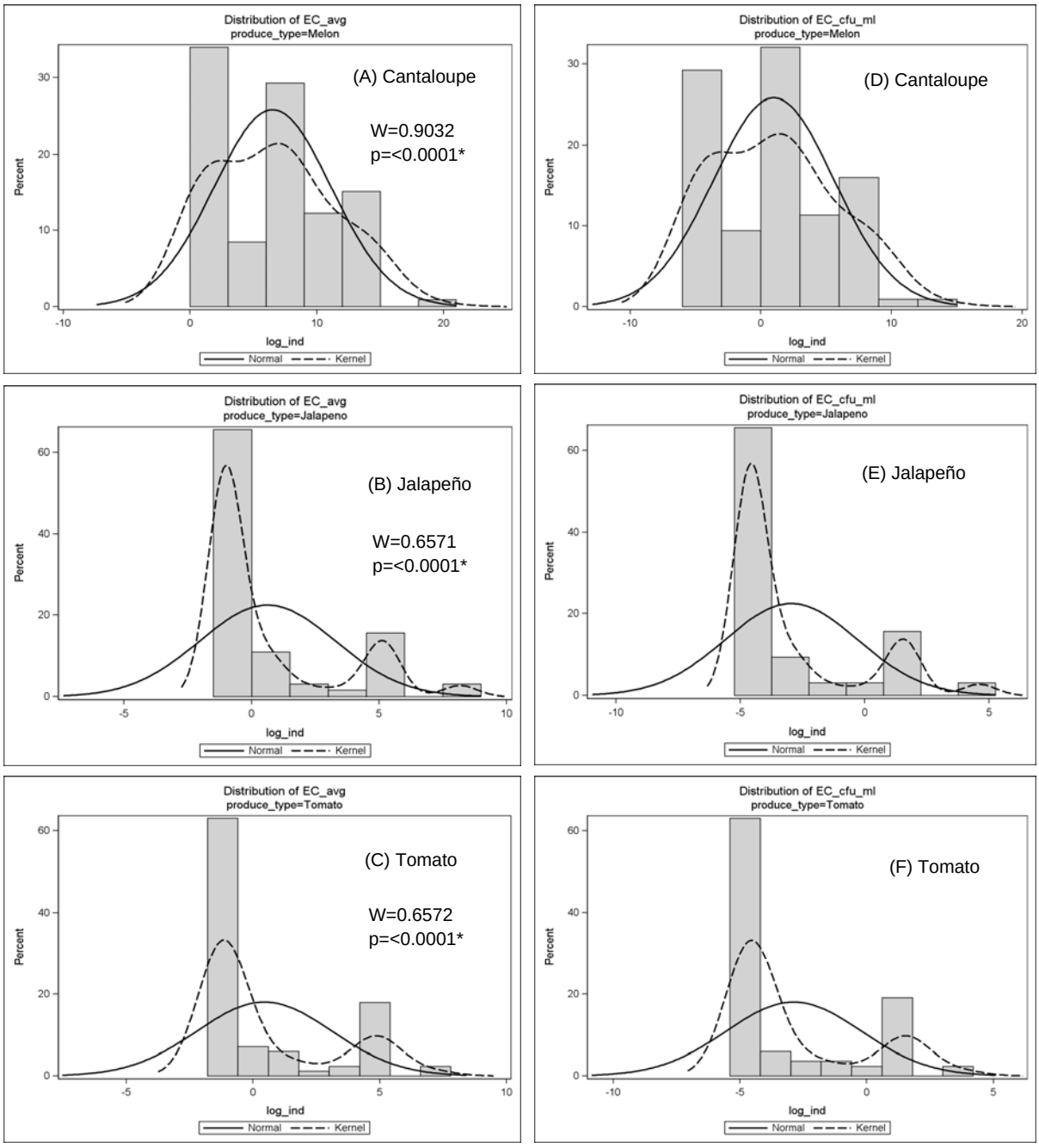

Figure 1B. Histograms illustrating *E. coli* distributions on each produce type and Shapiro-Wilk test results. Histograms A–C show concentrations (log_ind) in $\log_{10}$ CFU/fruit; D–F show concentrations in $\log_{10}$ CFU/ml. Dashed curve shows data distributions; solid curve shows normal distribution. Significant Shapiro-Wilk test statistic, W is indicated by p-value with asterisk (α=0.05) and indicates a non-normal distribution.

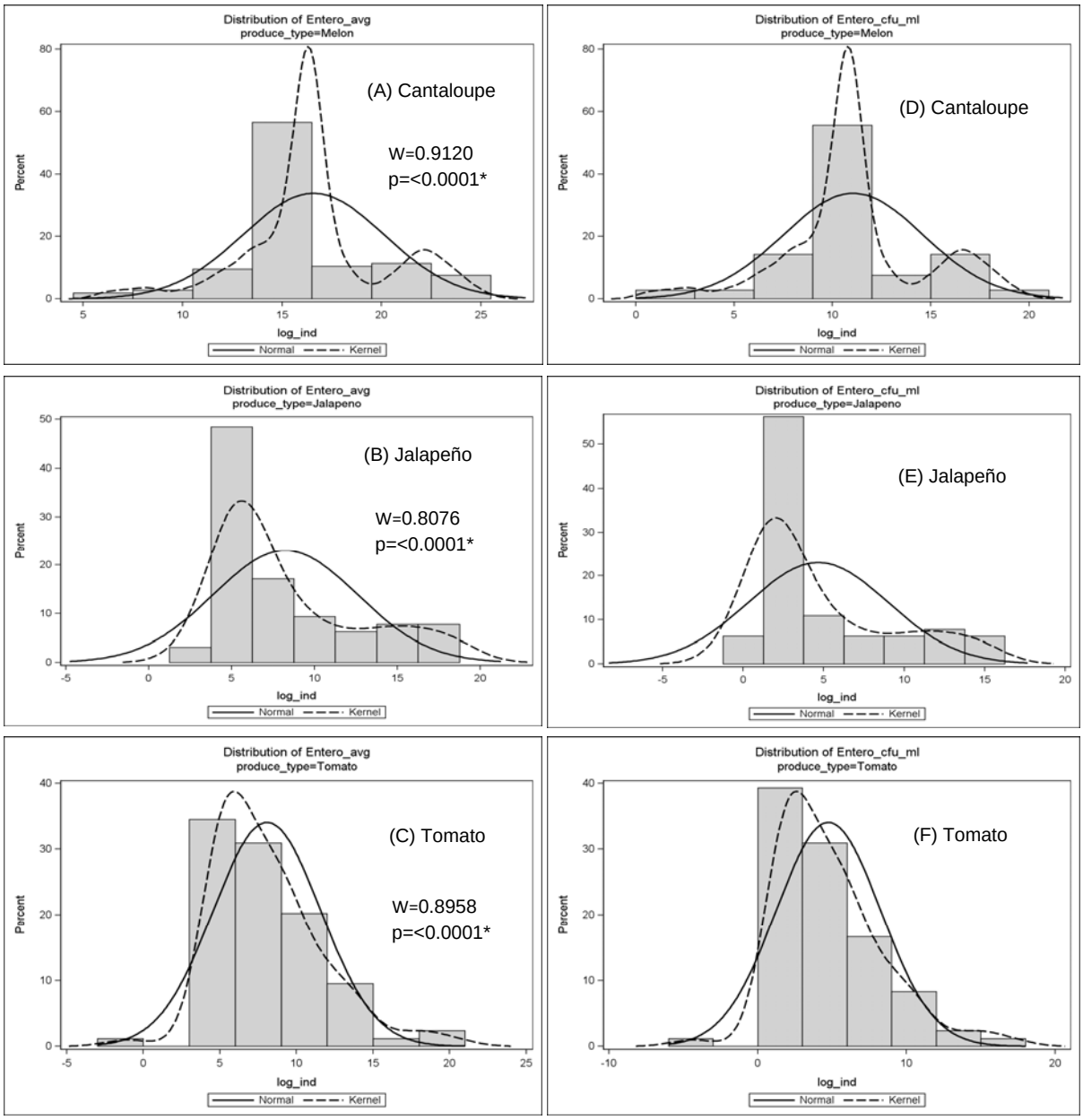

Figure 1C. Histograms illustrating *Enterococcus* distributions on each produce type and Shapiro-Wilk test results. Histograms A–C show concentrations (log_ind) in $\log_{10}$ CFU/fruit; D–F show concentrations in $\log_{10}$ CFU/ml. Dashed curve shows data distributions; solid curve shows normal distribution. Significant Shapiro-Wilk test statistic, W is indicated by p-value with asterisk (α=0.05) and indicates a non-normal distribution.

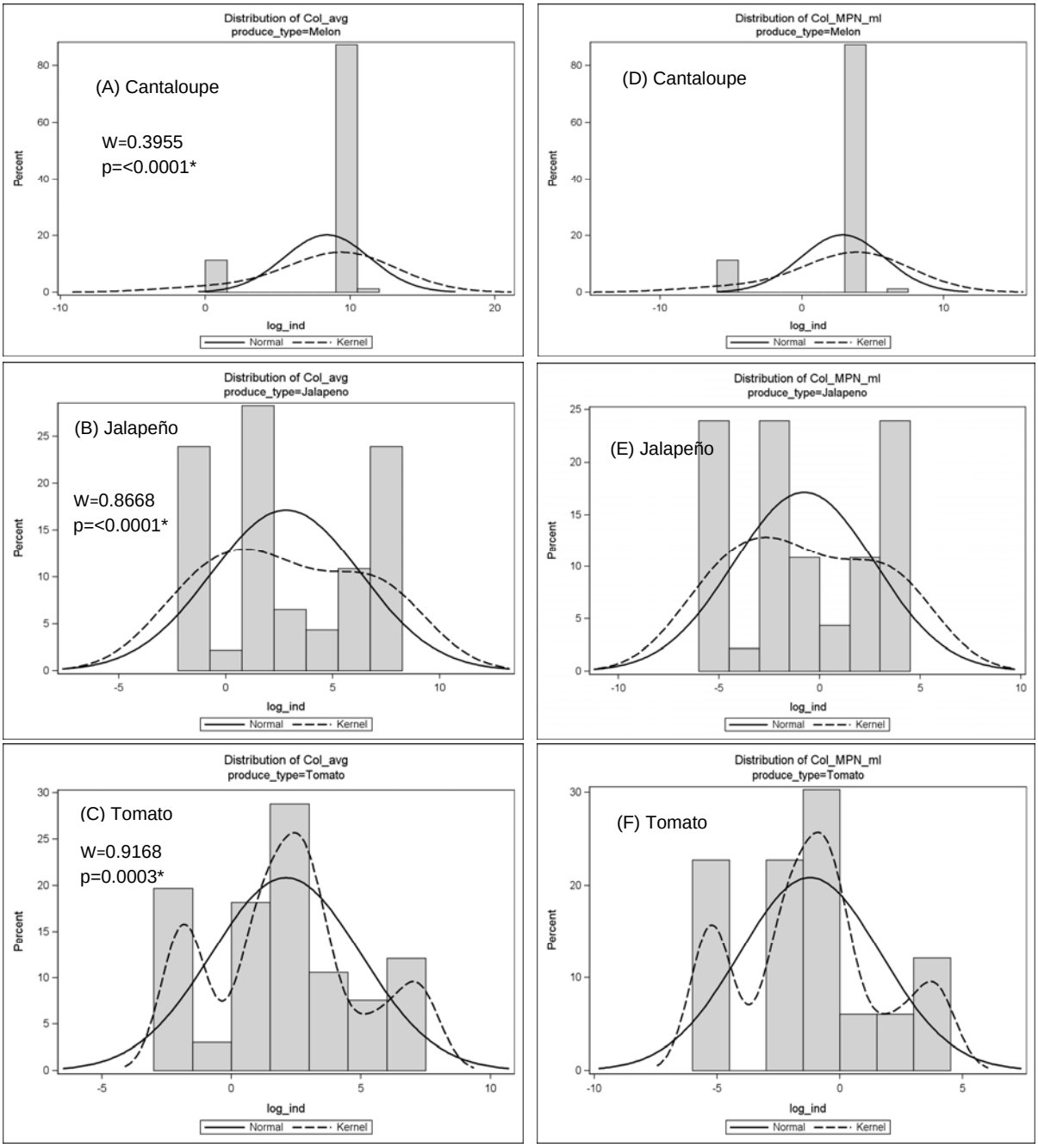

Figure 1D. Histograms illustrating somatic coliphage distributions on each produce type and Shapiro-Wilk test results. Histograms A–C show concentrations (log_ind) in $\log_{10}$ MPN/fruit; D–F show concentrations in $\log_{10}$ MPN/ml. Dashed curve shows data distributions; solid curve shows normal distribution. Significant Shapiro-Wilk test statistic, W is indicated by p-value with asterisk (α=0.05) and indicates a non-normal distribution.

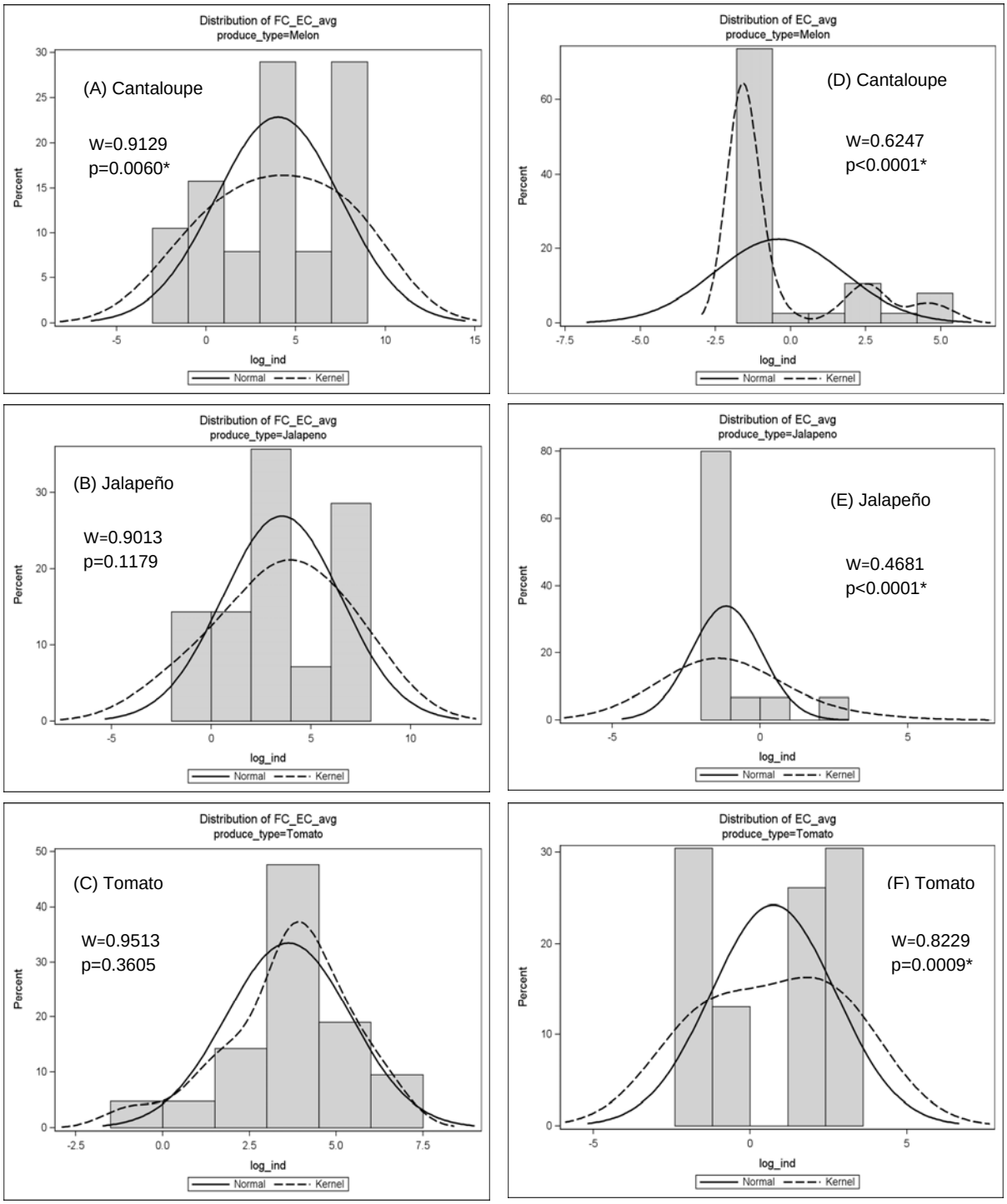

Figure 2A. Histograms illustrating fecal coliform (A–C) and *E. coli* (D–F) distributions on produce associated irrigation water and Shapiro-Wilk test results. Concentrations (log_ind) are measured in $\log_{10}$ CFU/100 ml. Dashed curve shows data distributions; solid curve shows normal distribution. Significant Shapiro-Wilk test statistic, W is indicated by p-value with asterisk (α=0.05) and indicates a non-normal distribution.

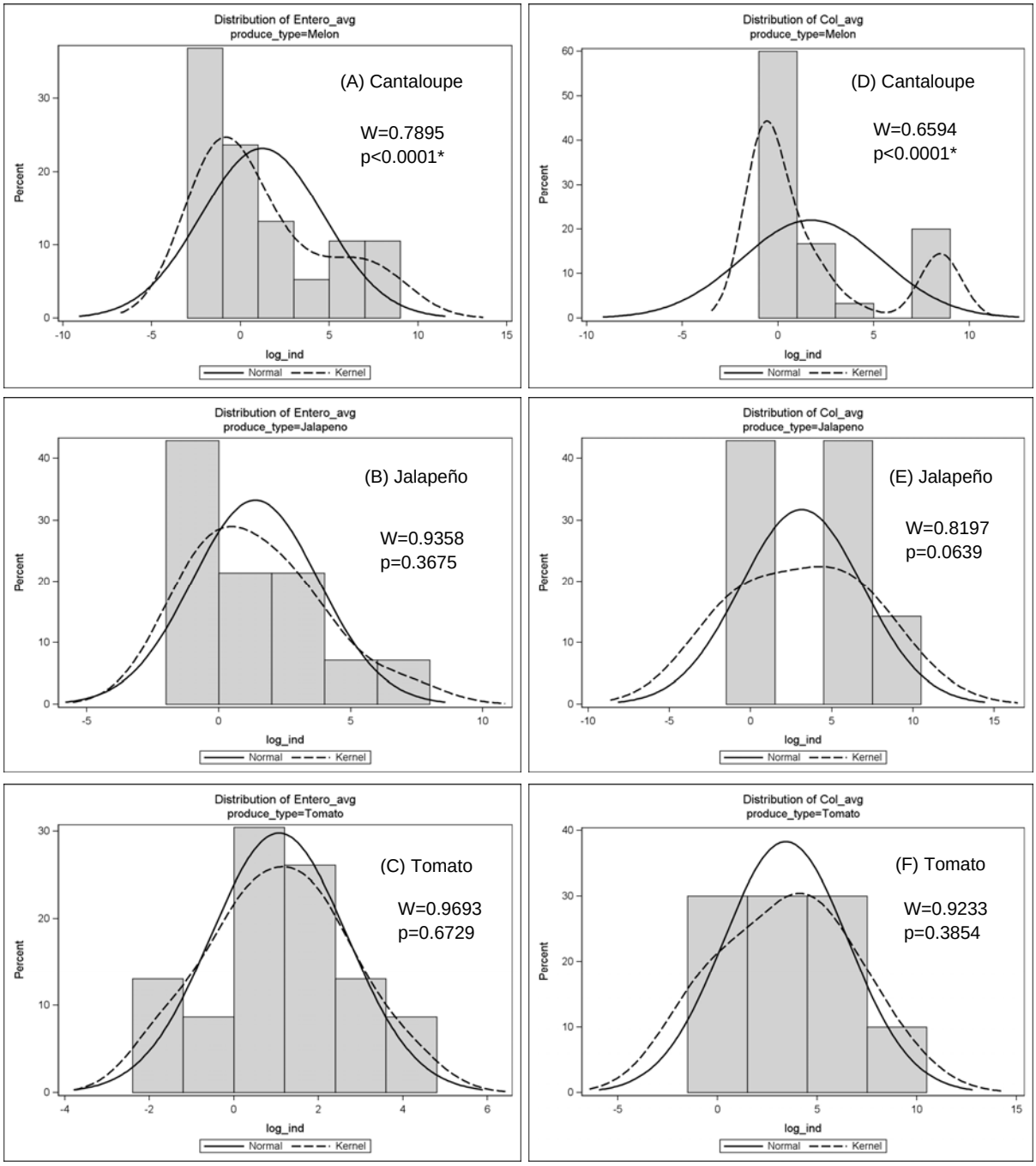

Figure 2B. Histograms illustrating *Enterococcus* (A–C) and somatic coliphage (D–F) distributions on produce associated irrigation water and Shapiro-Wilk test results. Concentrations (log_ind) are measured in $\log_{10}$ CFU or MPN/100 ml. Dashed curve shows data distributions; solid curve shows normal distribution. Significant Shapiro-Wilk test statistic, W is indicated by p-value with asterisk (α=0.05) and indicates a non-normal distribution.