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MICROBIAL WATER QUALITY—MONITORING AND MODELING

Spatiotemporal Variability in Microbial Quality of Western US Agricultural Water Supplies: A Multistate Study

Melissa L. Partyka,* Ronald F. Bond, Jennifer A. Chase, and Edward R. Atwill

Abstract

In 2011, the US Congress passed the Food Safety Modernization Act, which tasks the US Food and Drug Administration to establish microbiological standards for agricultural water. However, little data are available for the microbiological quality of surface water irrigation supplies. During the 2015 irrigation season, we conducted a baseline study on the microbial water quality of large irrigation districts in California (n = 2) and Washington (n = 4). Monthly samples (n = 517) were analyzed for bacterial indicators (fecal coliforms, enterococci, and *Escherichia coli*) and pathogens (*Salmonella* spp., *E. coli* O157, and non-O157 Shiga toxin-producing *E. coli* [STEC]). Although there was a high degree of variability ($\mu \pm SD = 59.13 \pm 106.0$), only 11% of samples (56/517) exceeded 126 colony-forming units (CFU) 100 mL^{-1}, and only six samples exceeded 410 CFU 100 mL^{-1}. Two volumes of water were collected for pathogen analysis (1 L and 10 L); prevalence of *Salmonella* in 10-L samples (68/149) was nearly double of that found in 1-L samples (132/517). We found STEC during ~9% of sampling events (58/517); serotypes O26 and O45 were the most common at 31 and 26%, respectively. Pathogens were not associated with exceedance of the regulatory threshold, yet the odds of detecting *Salmonella* increased approximately threefold (odds ration [O.R.] = 3.14, $p < 0.0001$) for every log increase in turbidity. Microbiological outcomes were highly district-specific, suggesting drivers of water quality vary across spatiotemporal scales. The true risk of contamination of produce from irrigation water supplies remains unknown, along with the optimal monitoring strategy to improve food safety.

Core Ideas

- Western US irrigation water supplies rarely exceed FDA agricultural water standards.
- Indicator bacteria are not predictive of pathogen prevalence or concentration.
- Prevalence of pathogens in irrigation water is higher than previously reported.
- Odds of detecting *Salmonella* improved >270% with an increase in sample volume.
- More research is needed to provide science-based guidance to the produce industry.

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In the United States, foodborne outbreaks associated with consumption of raw produce have increased >6% (Sivapalasingam et al., 2004) over a 20-yr period from the mid-1970s to the mid-1990s, coincident with a nearly 25.5% increase in the consumption of fruits and vegetables (Cook, 2011). In 2015 alone, 23% of multistate outbreaks of foodborne illness were associated with vegetables (7/30) (CDC, 2017). Continued coverage of foodborne outbreaks in the media has increased both public familiarity with many enteric pathogenic bacteria (i.e., *Escherichia coli* O157, non-O157 Shiga toxin-producing *E. coli* [STEC], and *Salmonella* spp.) and scrutiny of the produce industry. In response to growing public concern over food safety, the US Congress passed the 2011 Food Safety Modernization Act, which commissioned the US Food and Drug Administration (FDA) to create rules aimed at reducing rates of foodborne outbreaks. One of these rules, the Produce Safety Rule was established to address potential issues in the production, harvesting, and handling of fresh produce (FDA, 2011). One part of the Produce Safety Rule requires that fresh produce growers monitor their irrigation water supplies for indicator *E. coli* to ensure that, over time, the average concentration of *E. coli* in their water supplies remains below 126 colony-forming units (CFU) 100 mL^{-1} (2.1 $\log_{10}$ *E. coli*) (geometric mean) and the majority of samples (~90%) fall below a statistical threshold value of 410 CFU 100 mL^{-1} (2.61 $\log_{10}$ *E. coli*). These are the same regulatory standards used by the USEPA to ensure safety during body-contact recreational activities (e.g., swimming and boating) (USEPA, 2012).

While there have been an abundance of studies to model bacterial survival kinetics (Chase et al., 2017, Salazar et al., 2017), transfer dynamics (Atwill et al., 2015), and preharvest risk factors (Pagadala et al., 2015) linked to raw produce consumption (Ibekwe et al., 2005; Pachepsky et al., 2016; Tian et al., 2012), relatively little is known about the potential risk inherent in agricultural water supplies.

Notwithstanding this gap in knowledge, irrigation water has long been regarded as an important vehicle for pathogens associated with foodborne illnesses (Uyttendaele et al., 2015). According to the Centers for Disease Control and Prevention,

Western Center for Food Safety, Univ. of California, Davis, Veterinary Medicine 3B, 1089 Veterinary Medicine Dr., Davis, CA 95616. Assigned to Associate Editor Kyunghwa Cho.

Abbreviations: CFU, colony-forming units; FDA, Food and Drug Administration; LV, large volume; MFE, membrane-filtration-enrichment; MPN, most probable number; O.R., odds ratio; STEC, Shiga toxin-producing *Escherichia coli*; SV, small volume.

outbreaks that occurred during 2013 to 2014 from drinking water systems solely supplied by surface water accounted for nearly 80% of reported cases (Allende and Monaghan, 2015; Benedict et al., 2017; Uyttendaele et al., 2015). However, there is a paucity of research capable of linking microbial contamination in irrigation water to foodborne outbreaks in the United States (Pachepsky et al., 2016; Partyka et al., 2018). One possible explanation is that many irrigation water supplies, particularly those in the arid west, are controlled by irrigation districts, semigovernmental agencies, which can lead to reduced access to irrigation water supplies by researchers. With restricted access, researchers generally choose monitoring locations that are publicly accessible, which can limit their ability to understand how water quality fluctuates throughout the water delivery continuum, from source to point of application. This full breadth of information is necessary if we are to determine the actual risk associated with irrigation water supplies and design monitoring programs that are effective at reducing that risk. However, the question of what to monitor for continues to be debated.

Fecal indicator bacteria and bacterial pathogens are among the most commonly named causes of contamination of surface water in the United States (Grant et al., 2011). Although ubiquitous, pathogenic bacteria are rare and may not have a uniform distribution in surface water systems (Barker-Reid et al., 2009; Field and Samadpour, 2007), making it particularly challenging to capture or predict the variability of pathogens in moving surface water. Bacterial indicators continue to be regarded as the best way of quantifying degraded water quality, even as decades of research has shown that indicators are unreliable predictors of microbial pathogens (Nevers and Whitman, 2010; Pachepsky et al., 2016; Wade et al., 2003). Regardless of the debate among academics, the FDA codified the use of bacterial indicators as a means of monitoring agricultural water supplies. Specifically, they used the 2012 USEPA water quality standards for recreational contact and require produce growers to test their irrigation water source for *E. coli* at a prescribed rate, based on their source and application methods (FDA, 2011). However, there is no baseline understanding of the microbial quality of irrigation supplies along the water delivery continuum in the United States, and even less information on how irrigation waters may affect rates of foodborne illness associated with produce consumption.

The purpose of our study was to establish baseline microbial water quality data using Food Safety Modernization Act agricultural water criteria for irrigation districts using surface water systems. We wanted to better understand the association of spatial-temporal factors that influence microbial water quality variability to provide a foundation of baseline data and direction to stakeholders and policymakers as the criteria continue to be debated. In this manner, this study provides valuable feedback and direction for monitoring and managing microbial water quality within irrigation districts of the western United States.

Materials and Methods

Site Description and Sampling Locations

This baseline study was conducted during the irrigated growing season of 2015 in northern California and central Washington State (Fig. 1), within six large irrigation districts (hereafter referred to as districts), four in Washington (WA-1 to WA-4) and two in California (CA-5 and CA-6). Access to district-controlled irrigation networks was achieved following several months of negotiation and agreements of confidentiality. The participating districts primarily deliver water intended for agricultural uses but also sell to industrial and residential customers. Water laws in western states govern the timing and

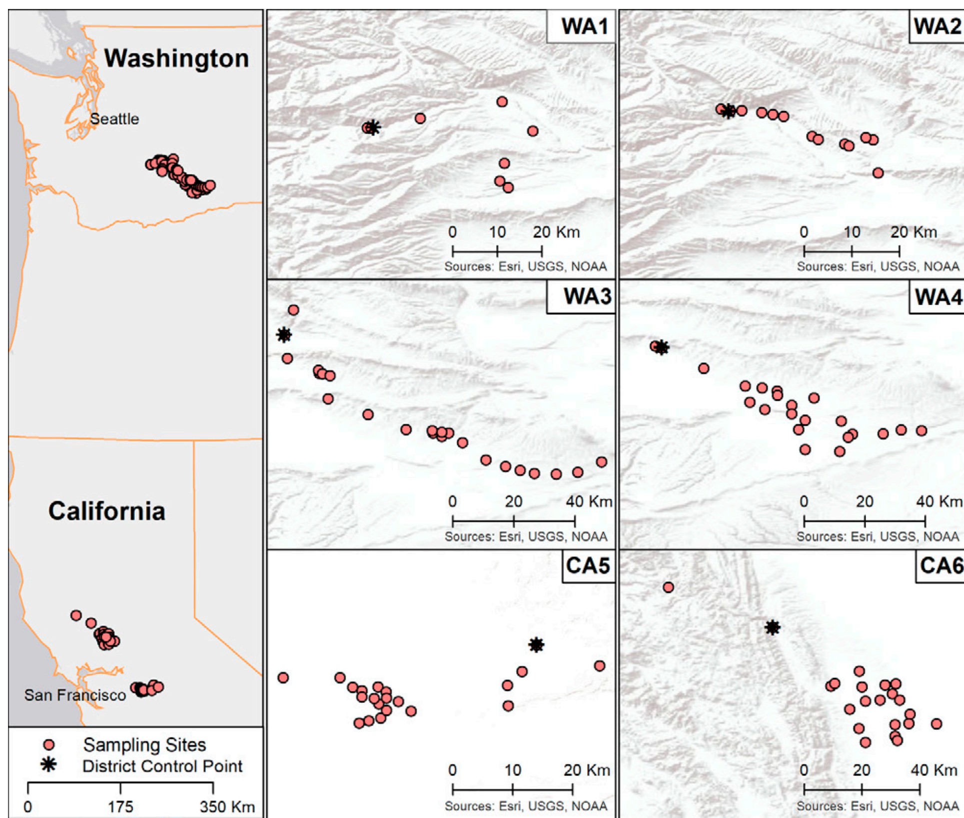

Fig. 1. Maps of sampling locations within participating irrigation districts of California (CA) and Washington State (WA). Place names and geographic characteristics have been removed for the purpose of confidentiality. Black asterisk on top of sampling location indicates location where water is diverted from a river and brought under control by an irrigation district.

abundance of water allocated to districts; most districts do not receive their allocation until the wet season has passed, typically between March and May. Water rights holders may have their allocations reduced by state or federal water resources management, particularly in times of drought.

Although all participating districts operate with varying combinations of open surface canals and pressurized pipes, one district, WA-1, delivered all water through a closed piped system after being diverted from an open water source (Supplemental Table S1). The surface water canals included in this study were constructed of various materials, including concrete, formed earth, geofabric, rip rap, or, as was common, a combination of materials. The studied irrigation districts in California delivered water primarily to tree-nut operations (walnut [*Juglans* L.], almond [*Prunus dulcis* (Mill.) D.A. Webb], and pistachio [*Pistacia mexicana* Kunth]) and processing tomatoes (*Lycopersicon esculentum* Mill.), whereas studied districts in Washington delivered mainly to tree-fruit operations (apple [*Malus* Mill.], pear [*Pyrus communis* L.], and cherry [*Prunus* spp.]) and hops (*Humulus lupulus* L.).

Water sampling sites (California [n = 42], Washington [n = 66]) were strategically chosen to represent the spatial extent of each district. The total study area encompassed by the six districts totaled ~1214.1 km^2 (>300,000 ac) of irrigated lands (Supplemental Table S1), an area 10 times the size of the city of San Francisco. The districts included in this study were diverse but shared many similarities; water was sourced from rivers or streams that flowed through undeveloped lands before being diverted into district canals, at which point water was channelized into large main canals before being subsequently diverted into smaller lateral canals. Sampling sites were stratified to include three main regions of the water delivery continuum: source water upstream of district control, the point of diversion where the district takes control, and irrigation delivery points within the district. The sites within the district were further classified as being along the main canal or within open or piped lateral canals.

Sample Collection

Monthly water monitoring took place from early May through late September 2015, the extent of water deliveries provided by participating districts during a year of extreme drought. At each sampling location, we collected two water samples (500 mL and 1 L) in sterile polypropylene Nalgene bottles (ThermoFisher Scientific) at an approximate depth of 0.3 m below the water surface. Water samples from piped locations were obtained at district-controlled filtration stations (spigot or valve); these sample points were flushed, cleaned with 70% ethanol (v/v), and flushed again prior to collection. To increase our ability to detect rare pathogens, additional large-volume (LV) samples were collected at ~25% of sites within each district at every sampling visit. These LV sites were chosen randomly a priori to each sampling visit (n = 149). All samples were placed on ice in coolers (small-volume [SV]) or in hard-walled bins (LV) and maintained at ~4°C during transport.

For each water sample, additional in situ physiochemical measurements (dissolved oxygen, pH, conductivity, temperature, and turbidity) were collected along with GPS location using a YSI ProDSS handheld multiparameter meter (Yellow Springs Instruments, Inc.). In situ meteorological measurements (wind speed and direction and air temperature) were measured using a Kestrel 5000 handheld anemometer (Nielsen-Kellerman). Canal and land use characteristics were recorded at each site during the first sampling visit and were verified before the close of the study period (Supplemental Table S2). To reduce intersample variability at point of collection, all samples were collected by the same researcher throughout the study.

Indicator Bacteria (*E. coli,* Fecal Coliforms, and Enterococci)

The 500-mL water samples were processed within 8 h of collection using the membrane filtration technique previously described by Partyka et al. (2018) for three bacterial indicators, including: *E. coli*, enterococci, and fecal coliforms. Filtered volumes were adjusted regularly based on prior knowledge of each source (previous bacterial concentrations) and visual inspection for turbidity and/or algae that could clog filters and reduce bacterial growth. Enumerated results were adjusted and recorded as colony-forming units per 100 mL. Media quality control procedures were conducted with every batch produced and validated according to the manufacturer's instructions.

Pathogenic Bacteria (*Salmonella, E. coli* O157, and Non-O157 Shiga Toxin-Producing *E. coli*)

The LV samples were processed using ultrafiltration methods (Partyka et al., 2018) with a final reduced volume (retentate) of approximately 1 L (10:1 concentration). Samples collected in California were received and processed by the laboratory staff within 8 h of collection, while Washington retentates were shipped overnight and processed within 30 h. The SV sample and approximately 500 mL of the retentate were analyzed for presence of *Salmonella* spp., *E. coli* O157 (O157), and non-O157 STEC using a membrane-filtration-enrichment (MFE) procedure (Supplemental Table S3). Up to six presumptive STEC isolates were analyzed for the six major serotypes associated with foodborne illness (O26, O45, O103, O111, O121, and O145) and tested for virulence genes (stx_1, stx_2, *eae*, and *hylA*) described by Paddock et al. (2012) and Paton and Paton (2002), respectively (Supplemental Table S3). The remaining portion of the retentate (~ 500 mL) was processed for quantification of *Salmonella* spp. using a most probable number (MPN)–MFE procedure with detection limits of 0.0015 to 1.4 MPN 1 mL^{-1} of retentate (1.65–1,540 MPN 100 mL^{-1} nonconcentrated water). All MFE samples were enriched, isolated on selective media, then confirmed with polymerase chain reaction as described by Kawasaki et al. (2005) and Atwill and Chase (2011) (Supplemental Table S3).

Statistical Analysis

Descriptive data and all statistical models were calculated using Stata 14 software (StataCorp LP). Microbiological and some environmental parameters were $\log_{10}$–transformed before analyses to conform with parametric assumptions. Within-group comparisons of environmental, categorical, and microbiological results were made using ANOVA followed by post-estimation using the Wald test for linear hypotheses. For complicated environmental data sets, we used a detailed model-fitting protocol fully described in Partyka et al. (2017). Briefly, data were evaluated for distribution shape, residual correlation structure, and autoregressive components before examining the presence of multilevel effects or interactions. We used a combination of criteria for determining

best model fits, including quasi-likelihood under the independence model criterion (QIC) (Cui, 2007), the Hausman test, and the Breusch-Pagan LM test (Rabe-Hesketh and Skrondal, 2012), and comparisons between a combination of Akaike information criterion (AIC) score and R^2 for final model selection. A univariate significance of $p \leq 0.10$ was used as a threshold for inclusion in preliminary model selection; robust variance estimation was then used to calculate p values within the finalized models to adjust for correlated data as a result of repeated measures. Spatial analyses and map generation were performed using ArcMap 10.5.1 (ESRI, Inc., Redlands, CA).

Results

Environmental Characteristics

Monthly sampling was conducted at 108 sites within six irrigation districts from May to September 2015 ($n = 517$). Small-volume samples were collected at every site; additional paired large-volume samples (LV) were collected from ~25% of sites during each sampling event ($n = 149$). The summer of 2015 fell during a multiyear drought, which led to decreased winter snowpack in the Cascade and Sierra Nevada Mountains and limited rainfall the proceeding spring. Consequently, many of the participating districts had lower-than-average allocations; two districts were forced to discontinue water deliveries either temporarily (WA-3) or permanently (CA-6) during the month of August. Physiochemical water quality varied greatly between states and districts within the same state (Table 1). California water samples were warmer than Washington samples, particularly at the start of the irrigation season, averaging 19.2°C in California during the first sampling event in May compared with just over 12°C for the Washington samples during the same period. Conductivity (µSm cm^{-1}), temperature (°C), pH, and $\log_{10}$ turbidity (NTU) were all significantly higher in CA-6 ($p < 0.0001$), while dissolved oxygen (mg L^{-1}) was lower ($p < 0.01$) in CA-6 compared with all other districts (Table 1). Median values of $\log_{10}$ turbidity were consistently >0.5 logs higher in CA-6, regardless of site or sampling event. These typical signs of degraded water quality are likely related to the fact that CA-6 is managed as a reclamation district, encouraging return flow to laterals from irrigated parcels. In Washington, average water temperature and $\log_{10}$ turbidity were significantly higher ($p < 0.0001$) in WA-3 and WA-4 compared with the other two Washington districts (Table 1); however, WA-4 had significantly ($p < 0.0001$) higher conductivity and lower pH than WA-3. Although WA-4 and WA-3 operate in the same geographic location, WA-4 is a junior water rights holder and diverts its water downstream of a confluence of two rivers and the discharge of other districts; this may explain the degraded water quality compared with the other Washington districts.

All districts included in the study had a mixture of land uses and canal construction materials. Orchards accounted for the highest proportion of adjacent land use (71/108), followed by residences (64/108), fodder crops (e.g., hay, corn [*Zea mays* L.], or alfalfa [*Medicago sativa* L.]) (38/108), and finally nondairy livestock (32/108). More than 47% of sites (51/108) were along earthen sided canals, while 59% (64/108) were downstream of bridges. Dairy operations were relatively rare, found within 500 m upstream of only 12 sites (11%) and only in three districts (WA-3, WA-4, and CA-5). As is typical for western growing regions, and particularly during the extreme drought of 2015, there was no measurable precipitation within the participating districts during this study, likely reducing the impact of surrounding land uses on local water quality.

Indicator Bacteria

Log_{10} concentrations of all indicator bacteria (*E. coli*, fecal coliforms, and enterococci) were highly variable (Table 1), with significant differences in each bacterium between states and irrigation districts ($p < 0.0001$) and across sampling events (p

Table 1. Summary statistics for indicator bacteria and physiochemical parameters within six participating irrigation districts in Washington and California.

Districts		Log_{10} EC†	Log_{10} FC†	Log_{10} EN†	Log_{10} turbidity	Temperature	pH	Dissolved oxygen	Specific conductivity
		——— CFU‡ 100 mL^{-1} ———			NTU	°C		mg L^{-1}	µS cm^{-1}
WA (n = 330)	mean (SD)	1.30 (0.58)	1.76 (0.66)	1.45 (0.51)	0.72 (0.31)	16.90 (3.95)	7.81 (0.4)	9.46 (0.92)	91.63 (58)
	min.–max.	0–2.93	0–3.86	0.15–2.57	0.14–1.82	6.20–25.30	6.90–9.01	7.33–13.75	1.0–483.1
WA-1 (n = 40)	mean (SD)	0.57 (0.37)	0.70 (0.46)	0.73 (0.29)	1.27 (0.1)	12.40 (4.5)	7.90 (0.5)	10.0 (0.8)	55.60 (14.7)
	min.–max.	0–1.56	0–1.70	0.15–1.15	1.18–1.45	6.20–19.9	7.30–8.52	9.21–11.48	40.80–73.0
WA-2 (n = 65)	mean (SD)	1.15 (0.41)	1.40 (0.49)	1.42 (0.35)	0.60 (0.30)	14.20 (3.10)	7.60 (0.30)	9.90 (0.80)	64.70 (23.70)
	min.–max.	0.34–1.84	0.34–2.21	0.34–2.02	0.21–1.74	8.60–19.8	7.20–8.20	8.41–11.71	2.80–105.0
WA-3 (n = 115)	mean (SD)	1.23 (0.57)	1.93 (0.50)	1.61 (0.42)	0.70 (0.30)	17.80 (3.80)	8.0 (0.50)	9.30 (0.80)	80.30 (21.70)
	min.–max.	0–2.93	0.41–2.92	0.48–2.55	0.14–1.82	9.50–24.80	7.20–9.01	7.76–11.71	1.0–109.40
WA-4 (n = 110)	mean (SD)	1.73 (0.36)	2.18 (0.38)	1.55 (0.53)	0.75 (0.25)	18.20 (3.20)	7.70 (0.40)	9.30 (1.0)	124.10 (81.80)
	min.–max.	0.48–2.60	1.32–3.86	0.40–2.57	0.14–1.51	10.70–25.30	6.90–8.70	7.33–13.75	2.90–483.10
CA (n = 187)	mean (SD)	1.62 (0.59)	2.35 (0.63)	1.78 (0.58)	1.01 (0.49)	21.81 (2.57)	8.11 (0.40)	8.75 (0.86)	206.94 (176.70)
	min.–max.	0–3.13	0–4.61	0.30–3.96	0.09–2.55	12.70–29.80	6.90–8.89	5.02–12.45	51.10–1019.30
CA-5 (n = 104)	mean (SD)	1.64 (0.53)	2.09 (0.49)	1.63 (0.48)	0.62 (0.25)	21.0 (2.40)	7.80 (0.40)	8.90 (0.70)	66.40 (48.0)
	min.–max.	0–2.76	0–3.52	0.48–2.60	0.09–2.12	12.70–23.80	6.90–8.54	6.13–10.97	51.10–330.90
CA-6 (n = 83)	mean (SD)	1.60 (0.65)	2.67 (0.64)	1.98 (0.63)	1.44 (0.28)	22.90 (2.40)	8.50 (0.20)	8.60 (1.0)	383.0 (107.30)
	min.–max.	0–3.13	1.18–4.61	0.30–3.96	0.95–2.55	13.80–29.80	8.0–8.89	5.02–12.45	200.60–1019.30

† EC = *E. coli*, FC = fecal coliforms, EN = enterococci.

‡ CFU, colony-forming units.

< 0.05) (Supplemental Fig. S1). A multiple linear regression with interactions between district and sampling event explained >50% of the variability in $\log_{10}$ *E. coli* (adjusted $R^2 = 0.51$; Supplemental Table S4). Although there was a high degree of district-specific variability, mean concentrations of $\log_{10}$ *E. coli* when averaged across districts increased from May to June, followed by a decrease from June to September (Fig. 2).

Indicator bacteria concentrations also varied by physical location relative to the point of district control. Samples collected in the rivers and creeks upstream of district control (source water) had significantly ($p < 0.05$) lower concentrations of $\log_{10}$ *E. coli* ($\mu \pm$ SD = 1.16 ± 0.59 CFU 100 mL^{-1}) than samples collected from within the constructed canals of the irrigation districts ($\mu \pm$ SD = 1.46 ± 0.59 CFU 100 mL^{-1}). Additionally, for samples taken within a district, Euclidean (straight-line) distance from point of district control (km) had a significant curvilinear (polynomial) relationship with concentration of $\log_{10}$ *E. coli* ($p < 0.0001$) (Supplemental Fig. S2). Average concentrations increased up to ~30 km and decreased as distances exceeded 40 km from the point of district control {$\log_{10}$ *E. coli* $= 1.16 + (0.017 \times \text{km}) + [(-3.33 \times 10^{-6}) * \text{km}^3)]$}. Owing to the strong influence of district and event-specific effects, no other physiochemical or environmental characteristics collected were capable of explaining the observed trends in indicator bacteria concentration and so were not included in any of the regression models.

Pathogens

Salmonella was isolated from nearly 26% of all SV samples (132/517) and more than 45% of all LV samples (68/149) (Table 2). The odds of detecting *Salmonella* improved more than 270% (odds ration [O.R.] = 2.78, $p < 0.001$) with an increase in volume from 1 L to 10 L in paired samples ($n = 149$). *Salmonella* was more commonly detected in sampling events from California (90/187) than from Washington (85/330), regardless of volume, although prevalence varied by district (Supplemental Table S5). Using a multiple-logistic regression model, we determined that district, collection of paired samples, sampling location within the irrigation network, and Euclidean distance from point of control were all significantly associated with the probability of detecting *Salmonella* (Table 3). According to the model, samples collected from CA-6 were nearly 27 times more likely to test positive for *Salmonella* than samples from WA-1 (O.R. = 26.99, $p < 0.003$). Location within the irrigation network was also a significant predictor ($p < 0.05$); samples collected from a main or lateral canal within a district were more than three times more likely to test positive for *Salmonella* compared with samples collected from the source water upstream of a district (O.R. = 3.19 and O.R. = 3.77, respectively). Even when district and location within the network were controlled for, there was a modest, yet highly significant ($p < 0.0001$) negative association between distance from the point of district control and *Salmonella* detection (Table 3). For every kilometer increase in distance down the canal network, the odds of detecting *Salmonella* decreased by ~3% (O.R. = 0.97). Detection of *Salmonella* was not associated with any physiochemical, biological, or environmental variables aside from turbidity. Log_{10} turbidity had a strong, positive, univariate association with the probability of detecting *Salmonella* (O.R. = 3.14, $p < 0.0001$) (Fig. 3); however, a limitation in our

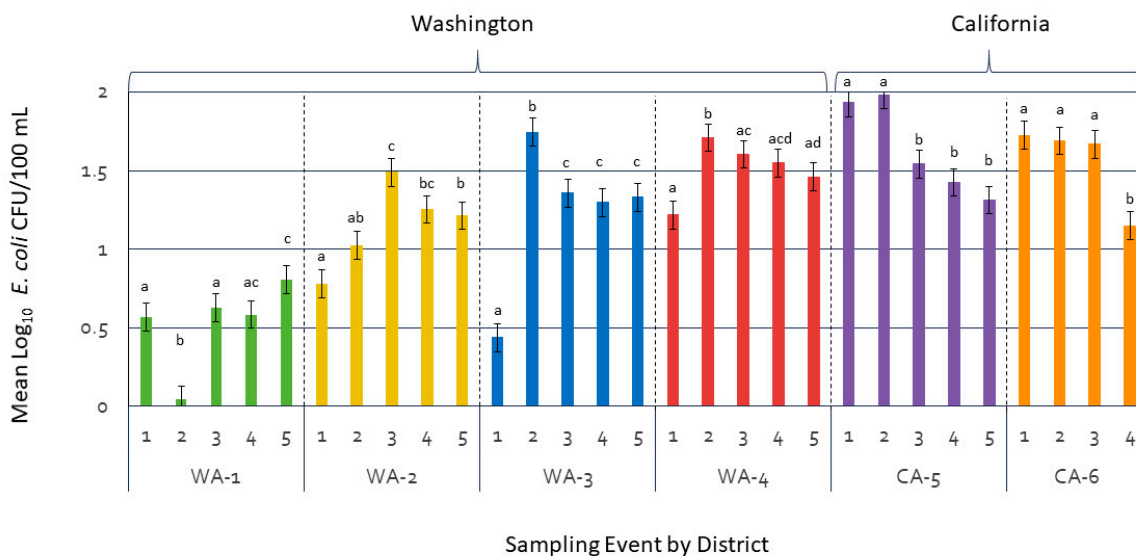

Fig. 2. Bar chart of average $\log_{10}$ concentrations of *E. coli* across districts by sampling event. Lowercase letters are used to denote statistical difference ($p < 0.05$) between sampling visits within each district. Letters are district-specific and do not describe relationships between districts for a given sampling event. CFU, colony-forming unit.

Table 2. Prevalence of bacterial pathogens (*Salmonella* spp., non-O157 Shiga toxin-producing *E. coli* [STEC], and *E. coli* O157, and in small-volume (SV = 1 L) and large-volume (LV = 10 L) samples collected in Washington and California during summer 2015.

Location	*Salmonella*		STEC		O157	
	SV	LV	SV	LV	SV	LV
	no. of positive samples (%)					
WA ($N_{SV} = 330$, $N_{LV} = 104$)	54 (16.4)	36 (34.6)	25 (7.6)	11 (10.6)	3 (0.9)	1 (1.0)
CA ($N_{SV} = 187$, $N_{LV} = 45$)	78 (41.7)	32 (71.1)	20 (10.7)	5 (11.1)	9 (4.8)	6 (13.3)
TOTAL ($N_{SV} = 517$, $N_{LV} = 149$)	132 (25.5)	68 (45.6)	45 (8.7)	16 (10.7)	12 (2.3)	7 (4.7)

Table 3. Multiple logistic regression model used to estimate the probability of detecting *Salmonella* spp. based on district, collection of paired small-volume (SV)–large-volume (LV) samples, location of sample within the irrigation network, and Euclidean distance from point of district control during the 2015 irrigation season in California and Washington.

	Coefficient	Odds ratio	*P*-value	OR 95% CI†
Intercept	–4.019	0.018	0.000	(0.002, 0.160)
District				
WA-1	0.000‡	1.000		
WA-2	1.865	6.456	0.097	(0.714, 58.384)
WA-3	2.427	11.326	0.031	(1.251, 102.542)
WA-4	1.667	5.296	0.142	(0.572, 49.059)
CA-5	2.352	10.501	0.037	(1.147, 96.177)
CA-6	3.296	26.991	0.003	(3.024, 240.921)
Paired SV-LV				
Not collected	0.000‡	1.000		
Collected	1.608	4.991	0.000	(3.101, 8.032)
Network location				
River (source)	0.000‡	1.000		
Canal	1.162	3.196	0.035	(1.087, 9.400)
Lateral	1.326	3.766	0.027	(1.161, 12.223)
Pipe	1.931	6.894	0.112	(0.636, 74.712)
Distance from district control (km)	–0.025	0.976	0.000	(0.962, 0.989)

† Confidence interval of the odds ratio.

‡ Referent category.

instrumentation prevented collection of turbidity data from piped systems and so it was excluded from the full model.

Salmonella was only enumerated in LV samples (n = 149) due to the volume constraints of our SV assay. The $\log_{10}$ mean concentration of *Salmonella* in positive LV samples was 2.52 (~316 MPN 10 L^{-1}), ranging between 2.08 and 3.89 (~119 to 7586 MPN 10 L^{-1}). All districts had higher *Salmonella* concentrations, on average, than WA-1 (Supplemental Fig. S3); however, only WA-2, CA-5, and CA-6 were significantly higher ($p < 0.001$), with the largest differences found between WA-1 and CA-6 ($\beta = 1.61$). District was the largest determinant of *Salmonella* concentration for our samples, accounting for more than 20% of the observed variability (adjusted $R^2 = 0.21$). Although $\log_{10}$ turbidity and $\log_{10}$ enterococci both had significant univariate relationships ($p < 0.05$) with $\log_{10}$ *Salmonella*, these associations were not significant when district was included; therefore, they were excluded from the final model for parsimony. No other physiochemical or environmental characteristics collected showed association with *Salmonella* concentration.

Using common conventions for classifying this group of bacteria, a sample was considered positive for STEC if any or all of the presumptive isolates (up to six) matched our targeted serotypes (O26, O45, O103, O111, O121, and O145) or if an isolate was positive for one or more of the four targeted virulence genes (*stx*$_1$, *stx*$_2$, *eaeA*, and *hylA*). A total of 157 isolates from 61 samples either were serotypeable for targeted STECs or contained at least one virulence gene (called "other"). Prevalence of STEC was similar between California and Washington, 12 and 10%, respectively; however, the prevalence of STEC in WA-1 (~22%) was more than twice that found in other Washington districts (Supplemental Table S5). An increase in sampled volume did not significantly improve our ability to detect STEC in either state ($p > 0.05$), although prevalence was marginally higher in LV samples (14/149) than in SV samples (44/517) (Table 2).

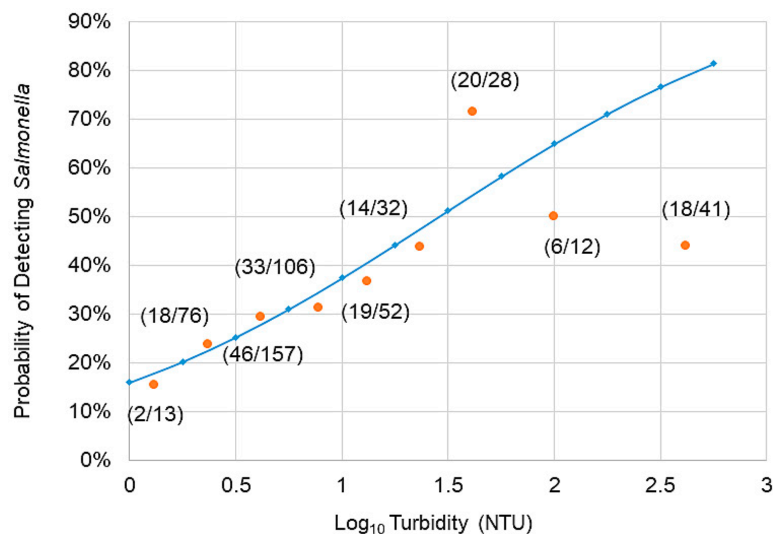

Fig. 3. Logistic regression model for the univariate association of $\log_{10}$ turbidity and the probability of detecting *Salmonella* spp. (solid line) plotted on top of actual *Salmonella* prevalence within binned ranges of $\log_{10}$ turbidity.

All serotypes included in our multiplex were detected at least once during the study. The serotype O26 was the most common, occurring in nearly one-third of all samples with detectable STECs (19/61) and accounting for 47 of 157 STEC isolates. Serotypes O45 and O145 were the second (16/61) and third (13/61) most common overall; however, serotype prevalence was different between California and Washington (Table 4). Of all STEC isolates, 62% (97/157) carried at least one virulence gene (Table 4). The hemolysin-producing gene (*hylA*) was the most common, detected in nearly 92% of all virulence positive isolates (89/97), followed by the intimin-producing gene (*eaeA*) (87/97), the gene for Shiga-like toxin I (*stx*$_1$) (61/97), and the gene for Shiga-like toxin

II (stx_2) (11/97) (Table 4). Serotype O103, although only the fourth most common serotype found in all samples, had a higher proportion of isolates with virulence (28/31) than the serotypes O26 and O145 combined (52/83). Of the 11 isolates that contained stx_2, 7 (64%) were classified as "other" as they were not serotypeable under our protocol. Upward of six isolates from presumptive STEC samples underwent serotyping; therefore, it was possible for multiple serotypes to be found in one sample. There were two instances where STEC positive samples of the same volume contained more than one serotype and one instance where different serotypes were found in a pair of SV–LV samples (Supplemental Table S6). One LV sample collected from WA-1 during July contained three different serotypes (O26, O103, and O145), all of which were positive for two or more virulence genes (Supplemental Table S6).

Escherichia coli O157 was detected during ~4% of sampling events (19/517) (Supplemental Table S5), with significantly higher prevalence in California (15/187) than Washington (4/330) (O.R. = 7.11, $p < 0.0001$). All positive samples in California were from within the same district, CA-5. The volume sampled was a significant factor in the detection of O157 in California; the odds of detecting O157 in LV samples from California (9/45) were threefold higher than in SV samples (O.R. = 3.04, $p = 0.047$). Interestingly, when comparing prevalence of O157 in paired SV-LV samples ($n = 149$), there were three instances where O157 was detected in SV samples but not in the matched LV sample. In fact, O157 was never detected in both SV and LV samples at the same time, suggesting that our probability of detecting this bacterium was driven by more than the volume sampled. Of the 19 samples that were O157 positive, 100% were also positive for three of the four targeted virulence genes (stx_2, *eaeA,* and *hylA*). Three samples, two from California and one from Washington, were also positive for stx_1.

Discussion

The FDA, through the creation of the Produce Safety Rule, has established regulations for the microbial quality of water supplies used to irrigate produce that is typically consumed raw (FDA, 2011). In simplified terms, the regulation requires that growers of commodities covered by the rule monitor their water supplies for indicator *E. coli* and ensure that, over time, the average concentration of *E. coli* in their water supplies remains below 126 CFU 100 mL^{-1} (2.1 $\log_{10}$ *E. coli*) (geometric mean) and the majority of samples (~90%) fall below the statistical threshold value of 410 CFU 100 mL^{-1} (2.61 $\log_{10}$ *E. coli*). However, at the time of the rule's creation, little information was available about the variability in concentrations of bacteria in typical irrigation water supplies, much less whether complying with the established standards would be possible for the majority of produce growers. The purpose of this study was to collect baseline data on the microbiological quality of water supplies in surface irrigation networks in two western states, California and Washington. We sought to evaluate the distribution and abundance of multiple indicator bacteria (*E. coli*, enterococci, fecal coliforms) and the prevalence of bacterial pathogens regularly associated with foodborne outbreaks (*Salmonella* spp., *E. coli* O157, and non-O157 STEC). In addition, we hoped to illuminate gaps in our knowledge of microbial water quality in irrigation water supplies to motivate future research throughout the water delivery continuum.

This study, to our knowledge, is the first of its kind to actively monitor multiple irrigation districts in more than one growing region during an entire produce irrigation season for indicators and pathogens. Concentrations of *E. coli* generally decreased in all districts from June to September (Fig. 2), suggesting a shared characteristic across geographic regions. This may be the result of changes within the source waters themselves; for example, during the late spring and early summer, there is a great deal of snow melt leading to overland flow and transport of soils and fecal sources into upstream water supplies. Management practices within surface irrigation networks may also contribute to this pattern, as districts must frequently treat or remove excess vegetation growing in canals during the summer months.

Single sample exceedances of the regulatory threshold were rare; only ~11% of all samples (55/517) had concentrations of *E. coli* higher than the geometric mean standard, and only six samples had concentrations greater than the statistical threshold value standard (1.1%). If we allow that the cumulative variability of all samples collected within a given district for the entire irrigation season may act as proxy for the potential variability in water quality at any given site within a district, then growers within these districts would all be within compliance of the current microbial standards. That is not to say that the irrigation water supplies sampled during this study pose no potential risk to food safety. One or more of the targeted pathogens were detected in 42% of all sampling events (219/517), and 34 events

Table 4. Distribution of non-O157 Shiga toxin-producing *E. coli* serotypes in samples collected from Washington and California with frequency of virulence in serotyped isolates.

Serotype	Samples		Isolates				
	WA	CA	Virulent isolates†	*hyla*‡	*eaeA*‡	*stx1*‡	*stx2*‡
	no.		no. of positive isolates (%)				
O26	12	7	38 (39.2)	35 (92.1)	38 (100)	29 (76.3)	0 (0)
O45	10	6	3 (3.1)	3 (100)	3 (100)	1 (33.3)	0 (0)
O103	8	3	28 (28.9)	28 (100)	21 (75)	20 (71.4)	1 (3.6)
O111	0	3	3 (3.1)	2 (66.7)	2 (66.7)	2 (66.7)	1 (33.3)
O121	1	0	1 (1)	0 (0)	0 (0)	0 (0)	1 (100)
O145	11	2	14 (14.4)	12 (85.7)	14 (100)	0 (0)	1 (7.1)
Other	0	5	10 (10.3)	9 (90)	9 (90)	9 (90)	7 (70)
All	36	25	97 (100)	89 (91.7)	87 (89.7)	61 (62.9)	11 (11.3)

† Prevalence of serotype within all isolates that were positive for at least one virulence gene.

‡ Prevalence of virulence gene within specific serotype.

(~7%) were positive for at least two pathogens. Yet, the average concentration of $\log_{10}$ *E. coli* in samples with multiple pathogens was only 1.59 (~38 CFU 100 mL^{-1}). We found no association between the concentration of indicators and prevalence or concentration of pathogens. In fact, the majority of samples that tested positive for any pathogen had $\log_{10}$ *E. coli* concentrations well below the regulatory standards (Fig. 4). This can hardly be considered surprising; multiple studies have examined the relationship between indicator bacteria and pathogens in surface waters, and although the results have been largely mixed, most studies do not show correlations (Pachepsky et al., 2016). Lack of correlation between indicators and pathogens is likely due to differentiation in the sources of these waterborne bacteria. For instance, indicator bacteria are known to establish environmental populations, while pathogens have been shown to decay more rapidly (Wilkes et al., 2009), suggesting more recent introduction into water supplies when detected. However, a number of dynamics are likely contributing to variability in microbial water quality within irrigation districts that need further exploration. For example, our participating districts use variable construction materials in their canals (e.g., earth, rip rap, and geofabric), vary in the proportion of open canal to closed pipe, and flow through highly variable landscapes (e.g., orchards, residential, and livestock) (Supplemental Table S1). In addition, they draw from different sources of water with their own unique history and chemical-physical qualities. Each combination of the multiple variables could lead to changes in bacterial sources and transport dynamics.

Little data are available for prevalence of pathogens in constructed irrigation networks; however, surveys of surface irrigation supplies in Pennsylvania (Draper et al., 2016) and the mid-Atlantic (Pagadala et al., 2015) found *Salmonella* in ~3 and 0%, respectively, in SV (<500-mL) samples, with no detectable O157 or STEC, much lower than found in SV samples of this study (Table 2). Sampling larger volumes has been shown to improve detection of rare pathogens (Jimenez and Chaidez, 2012; Polaczyk et al., 2008). For example, a 2014 monitoring study of six dammed reservoirs of central California found *Salmonella*, STEC, and O157 in 26, 9, and 1%, respectively, in 20-L samples (Partyka et al., 2018). Another survey of publicly accessible water of California's central coast by Cooley et al. (2014) found *Salmonella* in 65%, STEC in 11%, and O157 in 8% of Moore swabs exposed to water for >24 h, similar to the high prevalence we found in the California LV samples (Table 2). Yet prevalence alone is not necessarily indicative of potential risk to human health; it is important to also consider virulence and pathogenicity (Ahmed et al., 2009; Deer and Lampel, 2010).

We found 64% (39/61) of STEC positive samples were positive for at least one virulence gene and 34% (25/61) had three or more virulence genes associated with clinical diarrhea in humans (Paton and Paton, 1998). The frequency with which we isolated the hemolysin gene (*hylA*) (89/97 of isolates, 34/61 STEC positive samples; Table 4) was higher than previously reported in clinical, environmental, or animal isolates (Cabal et al., 2017; Halabi et al., 2008, Sidhu et al., 2013). Four STEC isolates and 16 of 19 O157 positive samples contained stx_2 without stx_1, a combination that has been linked to severe clinical outcomes such as hemolytic uremic syndrome (Paton and Paton, 1998). Further, six samples (10 isolates), presumptive for STEC, were not serotypeable using our protocol (Supplemental Table S3); however, all six of those samples were positive for at least one virulence gene, with six isolates carrying all four virulence genes. The presence of virulence-positive *E. coli* that do not belong to the "big six" group of non-O157 serotypes (USDA, 2011) is not unique in surface waters in California (Partyka et al., 2018), suggesting that there is the potential for currently unidentified STECs to rise to prominence in the future.

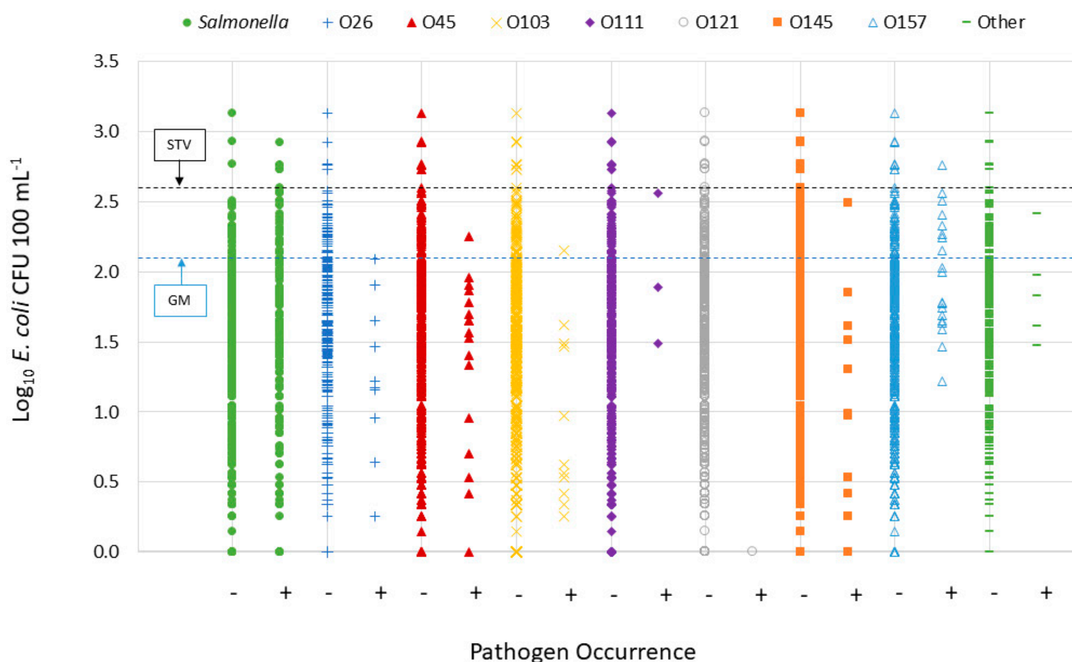

Fig. 4. Distribution of pathogens, *Salmonella* spp., *E. coli* O157, non-O157 Shiga toxin-producing *E. coli* serotypes as a function of log-transformed *E. coli* concentrations. On the X-axis, (–) symbol denotes samples where pathogens were not detected, (+) denotes given pathogen was detected. Geometric mean (GM) (blue dotted line) = 126 colony-forming units (CFU) 100 mL^{-1} or 2.1 $\log_{10}$ *E. coli* and statistical threshold value (STV) (black dotted line) = 410 CFU 100 mL^{-1} or 2.61 $\log_{10}$ *E. coli*.

Of further interest was the high prevalence of STEC-positive isolates from district WA-1 (41/157), our smallest district, with five of eight sites within a piped main canal. We repeatedly isolated two STEC serotypes (O145 and O103) with the same virulence profiles (*hylA* and *eaeA,* and *hylA, eaeA,* and *stx1,* respectively) in consecutive sites along the network during one sampling event: the river source, diversion point, the preliminary canal, and the first site in the fully pressurized (piped) main line. A third serotype, O26, was also isolated from an LV sample at the first piped site during the same visit (Supplemental Table S6). During the following sampling event, ~30 d later, identical isolates of O103 and O26 with the same virulence patterns were detected from piped sites further downstream. This seems to suggest that there was a source of virulent STEC in the upstream water supply that was diverted into the district and entered the piped mainline and remained there for an extended period of time (>30 d). We did not encounter this regularity in STEC occurrence or recurrence of identical virulence patterns in downstream samples in any other district. We speculate that enclosure of contaminated water into the protected environment of a piped water system allowed the potential of biofilm formation as described by Pachepsky et al. (2012) and Biscola et al. (2011), both of whom showed that under environmental or laboratory conditions, a variety of wild-type non-O157 STEC were able to form biofilms on constructed materials.

A great deal of research is clearly still required to understand not only the variability in microbial water quality of irrigation water supplies but also the potential risk to consumers of produce irrigated with contaminated water. We have continued over the past 2 yr to extend this research into additional irrigation districts, enlisting the support of landowners and growers to increase the spatial density of our sampling efforts; however, larger understanding of irrigation networks will require expansion into additional growing regions in multiple states. One of the many obstacles to research in surface water supplies is the difficulty that researchers face when attempting to gain access to irrigation districts. The Produce Safety Rule places the burden of compliance solely on growers, and most irrigation districts are answerable to a board of directors that are often liability and risk averse. This arrangement can make it particularly difficult to gain access for not only indicator but particularly pathogen sampling; <50% of the districts we approached agreed to participate. Those districts that did participate did so under strict confidentiality, which further limits the ability of others to perform comparative studies in the future. To expand our understanding of the topic and continue as impartial advocates for science-based decision making, it is incumbent upon the research community to engage with growers and irrigation districts on the value of collaborative research so that we may all benefit from the best science possible, not merely the best science available.

Conclusions

Scientific data for distribution and abundance of bacterial indicators and pathogens in surface irrigation water supplies are limited. The FDA Produce Safety Rule requires that growers of ready-to-eat commodities begin regularly monitoring their water supplies for indicator *E. coli* beginning in 2022; however, little data are available to determine whether this will lead to improvements in food safety. Yet, until epidemiological studies can be performed to estimate the risk to human health from the consumption of produce that has been irrigated with surface water of equivalent quality, we must rely on regular monitoring. While we found a high degree of spatial and temporal variability in concentrations of microbial indicators within and between participating irrigation districts, it appears that growers within these districts will not have difficulty in complying with regulatory standards of the Produce Safety Rule. However, we also detected one or more pathogens in >42% of samples, indicating the need for additional research to improve our understanding of these dynamic, manmade systems. This is the first study of its kind to regularly monitor irrigation water in multiple surface water irrigation districts, during an entire irrigation season, providing baseline data useful for future epidemiological research.

Supplemental Material

Supplemental content includes tables describing the physical/engineering characteristics of the participating irrigation districts (Supplemental Table S1), a complete list of covariates collected and considered in statistical models (Supplemental Table S2), and a description of the methodological approaches used for pathogen detection and confirmation (Supplemental Table S3). Supplemental Table S4 is the full statistical model for concentrations of $\log_{10}$ *E. coli,* while Supplemental Tables S5 and S6 describe the prevalence of pathogens across districts and the patterns of virulence in isolated STECs, respectively. Supplemental figures (S1–S3) illustrate the patterns of variability in three indicator organisms, fecal coliforms, enterococci, and *E. coli* over sampling event by district, the association of $\log_{10}$ *E. coli* with Euclidean distance along a canal, and the differences in distribution of $\log_{10}$ *E. coli* and $\log_{10}$ *Salmonella* spp. across districts.

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