Predicting *Salmonella* Populations from Biological, Chemical, and Physical Indicators in Florida Surface Waters

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Coliforms, *Escherichia coli*, and various physicochemical water characteristics have been suggested as indicators of microbial water quality or index organisms for pathogen populations. The relationship between the presence and/or concentration of *Salmonella* and biological, physical, or chemical indicators in Central Florida surface water samples over 12 consecutive months was explored. Samples were taken monthly for 12 months from 18 locations throughout Central Florida ($n = 202$). Air and water temperature, pH, oxidation-reduction potential (ORP), turbidity, and conductivity were measured. Weather data were obtained from nearby weather stations. Aerobic plate counts and most probable numbers (MPN) for *Salmonella*, *E. coli*, and coliforms were performed. Weak linear relationships existed between biological indicators (*E. coli*/coliforms) and *Salmonella* levels ($R^2 < 0.1$) and between physicochemical indicators and *Salmonella* levels ($R^2 < 0.1$). The average rainfall (previous day, week, and month) before sampling did not correlate well with bacterial levels. Logistic regression analysis showed that *E. coli* concentration can predict the probability of enumerating selected *Salmonella* levels. The lack of good correlations between biological indicators and *Salmonella* levels and between physicochemical indicators and *Salmonella* levels shows that the relationship between pathogens and indicators is complex. However, *Escherichia coli* provides a reasonable way to predict *Salmonella* levels in Central Florida surface water through logistic regression.

Water quality can be characterized by three types of analysis, index microorganisms, indicator microorganisms, and various physicochemical water characteristics. Indicator microorganisms are used to suggest, or “indicate,” the possible presence of fecal contamination (1). Index organisms represent the presence and behavior of a pathogen in a given environment (2), and one organism can be both an indicator and an index organism. Physicochemical water characteristics include turbidity, temperature, pH, and oxidation-reduction potential (ORP) (3). Physicochemical measurements have the advantage of being considerably more rapid than microbial-based measurements, but such measurements may not correlate with microbiological quality.

The United States produce industry currently relies on testing 100 ml of water for indicator organisms, specifically, generic *Escherichia coli*, as a means to monitor microbial water quality used for edible horticultural crop production (4–6). There are a wide variety of bacterial genera, groups, and species, viruses, and bacteriophages that have been used or proposed for use as indicator microorganisms (3, 7–9). Coliforms, either total or fecal, are a common choice of indicator organism. Total coliforms are aerobic and facultatively anaerobic, Gram-negative, non-spore-forming, rod-shaped bacteria that produce gas and acid upon lactose fermentation within 48 h at 35°C; fecal coliforms are a subset of coliforms that also ferment lactose at 44°C. Generic *E. coli* has been proposed as both an indicator and an index microorganism for enterohemorrhagic *E. coli*, *Salmonella* spp., and *Shigella* spp. (10).

When contaminated agricultural water comes into contact with the edible portion of a plant, for example, during irrigation, pesticide and fertilizer application, and/or frost protection, the safety of fresh produce items has been affected (11–13). In 2005, a large-scale outbreak of *Salmonella enterica* subsp. *enterica* serovar Newport from tomato consumption occurred, and the trace-back investigation revealed that the same serovar was in the irrigation

pond of the farm from which the suspect tomatoes were harvested (14). Another large outbreak in 2009 was epidemiologically linked to Serrano peppers, which came from a farm where a matching *Salmonella enterica* subsp. *enterica* serovar Saintpaul strain was isolated from the irrigation pond (15). Both outbreaks demonstrate that the quality of water applied to fresh produce is of utmost importance in ensuring food safety.

Contradictory results have been reported as to the efficacy of index or indicator organisms in predicting the presence and/or prevalence of human pathogens, such as *Salmonella*, in surface waters (16–19). While any specific pathogen may not be present in the sample being tested, the presence of index microorganisms is meant to suggest that pathogens have a reasonable likelihood of being present (1). The tests for index microorganisms are less cumbersome and time-consuming, as well as typically less expensive, than screening for the presence of individual pathogens. This makes the detection of index microorganisms a much more economical and practical choice than screening for individual pathogens.

Physicochemical water characteristics, such as turbidity, temperature, pH, and ORP, have all been used to monitor water quality in rivers and lakes (3), but the presence or absence of correlations between physicochemical measurements and microbial measurements of water is not discussed by the American Public Health Association (APHA) in their methods manual. Physical measurements like temperature and rainfall ($R^2 = 0.317$) have

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TABLE 1 Sampling site water source type and catchment area land usage

Site no.	Water source type	Immediate area land use(s)	% of watershed used for:		
			Agriculture	Rangeland	Urban/suburban
1	Lake	Agriculture, wetlands, residential	33	<1	29
2	Lake	Agriculture, wetlands	33	<1	29
3	Pond	Agriculture	33	<1	29
4	Creek	Wetlands	33	<1	29
5	Creek	Wetlands	33	<1	29
6	Stream	Agriculture	32	3	20
7	Creek	Agriculture	26	<1	49
8	Creek	Agriculture	32	12	25
9	Creek	Agriculture	32	12	25
10	Stream	Agriculture	32	12	25
11	River	Wetlands, agriculture	32	12	25
12	Creek	Agriculture	45	13	10
13	Canal	Agriculture	55	NA ^a	21
14	Canal	Agriculture	55	NA	21
15	Canal	Agriculture	55	NA	21
16	Canal	Agriculture	55	NA	21
17	Canal	Agriculture	55	NA	21
18	Canal	Agriculture	55	NA	21

^a NA, not available.

been correlated with *Salmonella* isolation frequency in seawater (20). The main advantage of monitoring a physicochemical characteristic is that nearly instantaneous results can be obtained and used to predict water quality so that a risk management decision can be made in a timely manner.

The relationship between *Salmonella* concentration and biological index organisms or physicochemical indicators in Central Florida surface waters over 12 consecutive months was explored.

MATERIALS AND METHODS

Water sampling. Eighteen surface water sites across Central Florida were sampled monthly for 12 consecutive months beginning in August 2010. Sites in rural agricultural areas, away from animal agriculture, including ponds, creeks, rivers, and canals, were selected. Sampling locations included two lakes, one pond, six creeks, two streams, one river, and six canals. The land use and catchment watershed for each site are detailed in Table 1.

Water was collected in 10-liter sterile carboys (Nalgene, Rochester, NY) fitted with 4 kg of lead weights attached to a rope. The rope was used to lower the mouth of the carboy to a depth of 20 cm into the water source. Samples from all but one location were collected away from the shore, using a bridge or other outcropping. Samples were collected on three separate days each month (six samples per day). All samples were taken before solar noon (the moment when the sun reaches its highest elevation on a given day at a given place) from a shaded area of the water source. Chemical and physical water characteristics were measured immediately after sampling as described below. Samples were transported to the laboratory and stored at 4°C for up to 24 h before microbial testing as described below. Samples were transported at ambient temperature, protected from the sun. No more than 4 h elapsed between sample collection and 4°C storage.

Chemical and physical water characteristics. Turbidity, temperature (air and water), pH, and ORP were measured. Each measurement was repeated in triplicate. Turbidity was measured in formazin attenuation units (FAU) using a portable colorimeter (DR/850; Hach Company, Loveland, CO) according to the manufacturer's instructions. Water and air temperatures were measured with a portable temperature probe (SH66A; Cooper Instrument Corporation, Middlefield, CT). The pH value and ORP were measured with a portable pH/ORP meter (pH 6 Acorn series; Oakton, Vernon Hills, IL).

Total aerobic plate count. Surface water samples were spread plated in duplicate onto tryptic soy agar (TSA; Difco, Becton, Dickinson, Sparks, MD) following serial dilutions in 0.1% peptone water (Difco, Becton, Dickinson, Sparks, MD) to determine total aerobic counts. Plates were incubated for 24 ± 2 h at 35°C ± 2°C, and all bacterial colonies were enumerated by hand.

Coliform/*E. coli* MPN. Colisure presence/absence snap packs (IDEXX Laboratories, Inc., Westbrook, ME) were used to determine coliform and *Escherichia coli* most probable numbers (MPN) in a five-by-three MPN configuration (10-, 1-, and 0.1-ml dilutions). Tubes were incubated for 24 h at 35°C ± 0.5°C. A yellow color indicated coliforms, and *E. coli* was identified by observing fluorescence using a 6-watt UV lamp. The MPN/100 ml was determined from the table in *Standard Methods for the Examination of Water and Wastewater*, 18th ed. (3).

Salmonella detection. *Salmonella* was determined in each water sample as previously described by McEgan et al. (21). Briefly, each water sample was concentrated using tangential flow filtration (TFF) to a final retentate volume of ca. 250 ml. A KrosFlo Research II pump (Spectrum Labs, Rancho Dominguez, CA) was used in combination with a Masterflex easy-load pump head (Spectrum Labs). The inlet flow rate was 1,000 ml/min. A MiniKros Plus tangential flow filter module (Spectrum Labs) made of polyethersulfone with a pore rating of 0.2 µm and a surface area of 1,050 cm² was used as a tangential flow filter. The TFF was run at a transmembrane pressure of 67 Pa. Double-strength lactose broth (250 ml; Difco, Becton, Dickinson) was added to the retentate as a preenrichment, stored for 1 h at room temperature, and incubated for 24 ± 2 h at 35°C ± 2°C.

Preenrichment was followed by an immunocapture of *Salmonella* using the Pathatrix system (Matrix MicroScience, Golden, CO). The method was modified such that the entire preenrichment was placed in a sterile stomacher bag (17.8 by 30.5 cm; Fisher Scientific, NJ) (instead of the typical 100-ml conical tube). Pathatrix *Salmonella* capture beads (50 µl) were added to the system and used according to the manufacturer's instructions in a preset 30-min cycle.

The beads (40 µl) were used in a DNA extraction using the MoBio UltraClean DNA kit (MoBio, Carlsbad, CA). The extracted DNA was used in real-time PCR using the MicroSeq *Salmonella* spp. detection kit (Applied Biosystems, Carlsbad, CA). The real-time PCR kit was used according to the manufacturer's instructions in a Bio-Rad CFX96 RealTime thermocycler.

TABLE 2 Correlation coefficients determined for log MPN *Salmonella*/100 ml versus each of the physical, chemical, and biological water characteristics recorded from each sampling by sampling site

<i>R</i> ² for log MPN <i>Salmonella</i> /100 ml versus:									
Site no.	<i>E. coli</i> (log MPN/100 ml)	Coliforms (log MPN/100 ml)	APC (log CFU/100ml)	Water temp (°C)	Air temp (°C)	pH	ORP (mV)	Turbidity (FAU)	Conductivity (μS/cm)
1	0.108	0.009	0.001	0.000	0.158	0.003	0.007	0.220	0.021
2	0.000	0.059	0.119	0.205	0.235	0.006	0.008	0.011	0.210
3	0.082	0.079	0.178	0.101	0.043	0.123	0.146	0.141	0.033
4	0.149	0.087	0.144	0.099	0.123	0.006	0.027	0.691	0.405
5	0.034	0.003	0.003	0.069	0.103	0.068	0.080	0.152	0.036
6	0.097	0.254	0.344	0.014	0.127	0.177	0.165	0.195	0.039
7	0.066	0.039	0.023	0.026	0.076	0.009	0.271	0.042	0.265
8	0.263	0.003	0.007	0.031	0.019	0.052	0.240	0.078	0.194
9	0.150	0.001	0.092	0.065	0.016	0.021	0.424	0.055	0.315
10	0.278	0.037	0.038	0.037	0.035	0.071	0.555	0.359	0.269
11	0.606	0.003	0.099	0.005	0.039	0.272	0.070	0.584	0.355
12	0.230	0.079	0.020	0.119	0.136	0.204	0.346	0.019	0.024
13	0.372	0.019	0.000	0.153	0.303	0.010	0.005	0.109	0.018
14	0.017	0.322	0.354	0.408	0.120	0.191	0.154	0.023	0.030
15	0.016	0.016	0.377	0.138	0.430	0.075	0.022	0.010	0.065
16	0.131	0.168	0.069	0.148	0.041	0.013	0.037	0.024	0.054
17	0.107	0.004	0.000	0.022	0.087	0.185	0.102	0.152	0.450
18	0.678	0.247	0.002	0.001	0.020	0.000	0.101	0.237	0.053
Overall	0.078	0.000	0.004	0.000	0.006	0.076	0.070	0.010	0.015

^a Values greater than 0.4 are in bold.

Modified MPN for *Salmonella*-positive water samples. A 50-ml retained sample of each water sample was removed prior to filter concentration by TFF. A modified *Salmonella* MPN method was used to determine the *Salmonella* concentration (22) for each retained sample. A three-by-three tube MPN was set up, and dilutions were as follows: 10 ml in 10 ml double-strength lactose broth, 1 ml in 9 ml single-strength lactose broth, and 0.1 ml in 9 ml single-strength lactose broth. These were incubated for 24 ± 2 h at 35°C ± 2°C. Selective enrichment was accomplished by transferring a 1-ml aliquot to tetrathionate broth (TT broth; Difco, Becton, Dickinson) and 0.1 ml to Rappaport Vassiliadis (RV) broth (Difco, Becton, Dickinson). The selective enrichment broths were incubated for 24 ± 2 h at 35°C ± 2°C and for 48 ± 2 h at 41°C ± 2°C, respectively. Ten microliters was streaked onto xylose lysine Tergitol 4 (XLT4) and CHROMagar *Salmonella* Plus and incubated for 24 ± 2 h at 35°C ± 2°C following enrichment. Colonies displaying typical *Salmonella* phenotypes were confirmed biochemically on lysine iron agar slants (LIA; Difco, Becton, Dickinson) and triple sugar iron agar slants (TSI; Difco, Becton, Dickinson).

One representative biochemically confirmed *Salmonella* colony from each plate was transferred to TSA and incubated for 24 ± 2 h at 35°C ± 2°C. One colony was transferred to tryptic soy broth and incubated for 24 ± 2 h at 35°C ± 2°C, and DNA was extracted using the MoBio Ultra-Clean DNA kit. *Salmonella* was genetically confirmed by PCR of the *invA* and *oriC* genes. The *invA* primers were GTGAAATTATCGCCACGTTTCGGCAA and TCATCGACCGTCAAAGGAACC, producing a PCR amplicon of 284 bp; the *oriC* primers were TTATTAGGATCGCGCCAGGA and AAAGAATAACCGTTGTTCAC, producing a PCR amplicon of 163 bp. Both primer sets were as described by Malorny et al. (23). All reagents were obtained from the Fisher exACTGene Complete PCR kit (Fisher Scientific, NJ), and primers were used at a concentration of 20 μM. The PCR mix was as follows: 34.75 μl of water, 5 μl of 10× PCR buffer, 1 μl of each primer, 0.25 μl of *Taq* DNA polymerase, and 5 μl of template DNA. The optimized PCR conditions were as follows: 3 min at 94°C melting, followed by 30 cycles of 30 s at 94°C, 30 s at 58°C, and 1 min at 72°C, and a final elongation of 5 min at 72°C. PCR amplicons were visualized on a 1.8% agarose gel with 0.5× Tris-borate-EDTA (TBE) buffer. Gels were run at 70 V for 90 min.

Weather data collection. Rainfall and solar radiation data were collected from the Florida Automated Weather Network (<http://fawn.ifas.ufl.edu/>).

The closest weather-recording station to each individual sampling site was determined, and the data were applied accordingly.

Statistics. Correlations were determined using JMP Pro 9. Multiple linear regression analysis of *Salmonella* versus all parameters was done using SAS 9.3 (SAS Institute Inc., Cary, NC) software. A *P* value of <0.05 was set as the condition for entry of any parameter into a model.

To determine the probability of *Salmonella* enumeration, binomial logistic regression analysis using SAS 9.3 was done with all the other parameters as independent variables. Binomial logistic regression assumes a response variable with two possible outcomes (e.g., *Salmonella* is present or *Salmonella* is not present). Since all water samples tested had *Salmonella* counts above the detection limit, the simple absence or presence of *Salmonella* was not able to be used as a criterion. To overcome this limitation, we modeled the probability of enumerating a *Salmonella* concentration exceeding a particular value. Six *Salmonella* levels were selected for modeling: 3, 5, 10, 15, 20, and 60 MPN/100 ml. Using the lowest concentration as an example, *Salmonella* observations were converted to a value of 1 if an observation was ≥3 MPN/100 ml and a value of 0 if an observation was <3 MPN/100 ml. The same procedure was repeated for 5, 10, 15, 20, and 60 MPN/100 ml. This binomial logistic regression analysis was done with the coded *Salmonella* data as the dependent variable and all the other variables (turbidity, conductivity, air temperature, water temperature, pH, ORP, coliforms, and *E. coli*) as independent variables.

RESULTS

A total of 202 samples were collected and analyzed over the 12-month sampling period. Fourteen samples were not obtained due to laboratory error or inaccessibility of the water source due to unrelated factors (e.g., nearby herbicide application precluded access due to investigator safety). The correlation coefficients for each characteristic versus log MPN *Salmonella*/100 ml are displayed in Table 2. When each *R*² value is calculated for individual sampling sites, 152 of 162 of the *R*² values fall below 0.4, indicating a lack of correlation. Each characteristic will be discussed further in the appropriate section below.

***Salmonella* MPN.** All 202 water samples were positive for *Salmonella*. The TFF immunocapture real-time PCR method-

FIG 1 Populations of *Salmonella* (●), *E. coli* (◆), and coliforms (+), reported in log MPN/100 ml (left axis), and aerobic plate counts (×), reported in log CFU/100 ml (right axis), for 18 Central Florida sites sampled monthly for a continuous 12-month period.

ology has a detection limit of ca. 1 CFU/liter in 10 liters (21); all 10-liter surface water samples collected had greater than 1 CFU *Salmonella*/liter.

Salmonella concentrations for each site by sampling date can be seen in Fig. 1; the limit of detection for the MPN method used is

0.3 log MPN/100 ml. The median *Salmonella* concentration was 0.79 log MPN/100 ml for all samples; the upper quartile was 1.2 log MPN/100 ml, the lower quartile was 0.48 log MPN/100 ml, and 139 of 202 samples had *Salmonella* concentrations below 1 log MPN/100 ml. The highest *Salmonella* concentration, 3.0 log

MPN/100 ml, was identified in July (Fig. 1); however, the month of sampling was not strongly correlated with the *Salmonella* concentration ($R^2 = 0.20$).

Coliform and *E. coli* MPN. Values for coliform and *E. coli* log MPN/100 ml can be seen in Fig. 1. Many of the coliform (128/202) and some of the *E. coli* (17/202) MPN were at or over the upper limit of detection (≥ 3.2 log MPN/100 ml) for the MPN method used. The median coliform concentration was ≥ 3.2 log MPN/100 ml, with the lower quartile at ≥ 2.7 log MPN/100 ml. *E. coli* had a median concentration of 1.7 log MPN/100 ml, an upper quartile of 2.4 log MPN/100 ml, and a lower quartile of 0.60 log MPN/100 ml. Neither coliform log MPN/100 ml ($R^2 = 0.004$) nor *E. coli* log MPN/100 ml ($R^2 = 0.078$) values were strongly correlated with the *Salmonella* MPN values (Table 2). However, at sites 11 and 18, *E. coli* log MPN/100 ml resulted in higher correlations ($R^2 = 0.606$ and $R^2 = 0.678$, respectively). Coliform and *E. coli* log MPN/100 ml were not strongly correlated ($R^2 = 0.364$) (Table 3) with each other; fecal coliforms were not evaluated.

Aerobic plate count. The values for each aerobic plate count for each site can be seen in Fig. 1. The median aerobic plate count was 3.7 log CFU/100 ml; the upper quartile was 4.0 log CFU/100 ml, and the lower quartile was 3.2 log CFU/100 ml. The two highest aerobic plate counts, 5.2 and 5.5 log CFU/100 ml (Fig. 1H and D, respectively), both occurred during the month of July. The aerobic plate count, in log CFU/100 ml, did not correlate with the *Salmonella* log MPN/100 ml ($R^2 = 0.004$) (Table 2).

Air and water temperature. Air and water temperatures are displayed in Fig. 2. Not surprisingly, recorded air and water temperatures showed the strongest correlation of any two variables examined ($R^2 = 0.680$) (Table 3). Neither the air nor the water temperature correlated with the *Salmonella* log MPN/100 ml ($R^2 = 0.000$ and $R^2 = 0.006$, respectively) (Table 2). However, when calculated on an individual-site basis, site 14 ($R^2 = 0.405$) and site 15 ($R^2 = 0.403$) *Salmonella* concentrations showed relatively higher correlations with water and air temperatures, respectively (Table 2).

pH, ORP, turbidity, and conductivity. The pH and ORP values by sampling site over time are displayed in Fig. 3; similarly, turbidity and conductivity by sampling site over time are displayed in Fig. 4. None of these physical water characteristics correlated with *Salmonella* log MPN/100 ml, as shown by the R^2 values in Table 2. However, when calculated by site, ORP had a relatively high correlation with the *Salmonella* concentration at site 9 ($R^2 = 0.424$) and site 10 ($R^2 = 0.555$) while turbidity had a relatively high correlation with the *Salmonella* concentration at site 4 ($R^2 = 0.691$) and site 11 ($R^2 = 0.584$) and conductivity had a relatively high correlation with the *Salmonella* concentration at site 4 ($R^2 = 0.405$) and site 17 ($R^2 = 0.450$). pH correlations were below 0.4 at all sites.

Rainfall and solar radiation. The average rainfall (in the previous day, week, or month) before sampling did not correlate with *Salmonella*, coliform, *E. coli*, or aerobic plate count levels (data not shown). Solar radiation was poorly correlated with *Salmonella*, coliforms, *E. coli*, and aerobic plate count levels (data not shown).

Multiple linear and logistic regression analysis. A multiple linear regression analysis with pH, ORP, conductivity, turbidity, water temperature, air temperature, coliforms, and aerobic plate count as independent variables and the *Salmonella* concentration as the dependent variable revealed that *E. coli* and solar radiation were the only variables that were significantly correlated with the

TABLE 3 Pearson product moment correlation coefficients and *P* values, determined between each of the physical, chemical, and biological water characteristics for all sampling sites and months combined

Water characteristic	Correlation coefficient (<i>P</i> value) between water characteristics ^a									
	Air temp (°C)	pH	ORP (mV)	Turbidity (FAU)	Conductivity (µS/m)	Coliforms ^b	<i>E. coli</i> ^b	APC ^c	Solar radiation (W/m ²)	<i>Salmonella</i> ^b
Water temp (°C)	0.825 (0.0000)	0.208 (0.0031)	0.0117 (0.8800)	0.144 (0.0418)	0.1300 (0.0707)	-0.0165 (0.8170)	-0.144 (0.0432)	0.0125 (0.8620)	-0.0330 (0.6410)	0.1210 (0.0946)
Air temp (°C)		0.0127 (0.8580)	0.0254 (0.7450)	0.1410 (0.0473)	0.225 (0.0017)	0.0307 (0.6680)	-0.0744 (0.2990)	0.0297 (0.6790)	-0.0696 (0.3250)	0.0538 (0.4590)
pH			0.1330 (0.0867)	-0.0686 (0.3370)	0.423 (0.0000)	-0.0479 (0.5040)	-0.0141 (0.8450)	0.0398 (0.5790)	-0.0101 (0.8860)	0.0506 (0.4880)
ORP (mV)				-0.0209 (0.7880)	-0.0566 (0.4720)	0.0799 (0.3020)	-0.0499 (0.5200)	0.0151 (0.8450)	0.1010 (0.1870)	-0.0578 (0.4640)
Turbidity (FAU)					-0.0878 (0.2210)	-0.172 (0.0151)	-0.0297 (0.6780)	0.0336 (0.6390)	-0.0819 (0.2460)	0.0824 (0.2570)
Conductivity (µS/m)						-0.0592 (0.4130)	-0.317 (0.0000)	0.0568 (0.4320)	-0.273 (0.0001)	-0.1210 (0.0994)
Coliforms ^b							0.364 (0.0000)	0.212 (0.0029)	-0.0670 (0.3450)	0.0032 (0.9650)
<i>E. coli</i> ^b								0.0386 (0.5920)	0.151 (0.0330)	0.248 (0.0005)
APC ^c									-0.0132 (0.8520)	0.165 (0.0216)
Solar radiation (W/m ²)										

^a Coefficients displayed in bold are statistically significant ($P < 0.05$).

^b *E. coli*, coliforms, and *Salmonella* are reported in log MPN/100 ml.

^c Aerobic plate counts (APC) are reported in log CFU/100 ml.

FIG 2 Air (○) and water (●) temperatures, reported in °C, measured using a temperature probe at the time of sampling for 18 Central Florida sites sampled monthly for a continuous 12-month period.

Salmonella concentration ($P < 0.05$); however, the resulting R^2 was very low (<0.1). Table 3 shows Pearson product moment correlation coefficients and P values for all the parameters. Correlation coefficients closer to 1 or -1 indicated a strong linear relationship between variables, similar to the relationship indicated by R^2 (24). Positive correlation coefficients indicate that the

two variables are positively correlated, while a negative correlation coefficient indicates the opposite. Table 3 shows that *E. coli* and solar radiation levels were positively correlated ($P < 0.05$) with *Salmonella* concentrations but with low correlation coefficients.

Given the absence of a strong linear relationship between *Salmonella* concentrations and any variable investigated, multiple

FIG 3 pH (○, left axis) and ORP (●, right axis), measured at the time of sampling for 18 Central Florida sites sampled monthly for a continuous 12-month period.

logistic regression analysis was used to predict the probability of the *Salmonella* concentration exceeding a given concentration as a function of any of the variables investigated. *Escherichia coli* concentration was the only variable that was significantly correlated

with the probability of the *Salmonella* concentration exceeding a certain value ($P < 0.05$). A model relating the probability of *Salmonella* occurring at levels of $\geq 3, 5, 10, 15, 20$, or 60 MPN/100 ml was developed. The model has the form of $\logit P = C \pm P$, C

FIG 4 Turbidity (●; left axis) and conductivity (○; right axis), measured at the time of sampling for 18 Central Florida sites sampled monthly for a continuous 12-month period.

FIG 5 Logistic regression model for predicting the probability of enumerating ≥ 3 MPN *Salmonella*/100 ml (solid line; $P = 0.815$), 5 MPN/100 ml (long dashes; $P = 0.035$), 10 MPN/100 ml (short dashes; $P = 0.014$), 15 MPN/100 ml (dash and two dots; $P = 0.010$), 20 MPN/100 ml (dash and one dot; $P = 0.009$), and 60 MPN/100 ml (dotted; $P = 0.020$).

is a constant, and K is the model coefficient. Individual plots for each criterion used to predict the probability of occurrence of *Salmonella* given any level of *E. coli* in surface water are shown in Fig. 5. With higher *E. coli* concentrations in surface water, the probability of enumerating *Salmonella* at any of the six concentrations (3, 5, 10, 15, 20, and 60 MPN/100 ml) rises in a roughly proportionate manner from the lowest level of *E. coli* observed (-1 log MPN/100 ml) to the highest (3.2 log MPN/100 ml).

The probabilities of *Salmonella* exceeding a particular value are arrayed in an essentially logical manner. For example, when the *E. coli* concentration is 1 log MPN/100 ml, the model predicts an 80% chance of enumerating ≥ 3 MPN *Salmonella*/100 ml, a 43% chance of enumerating ≥ 5 MPN *Salmonella*/100 ml, a 24% chance of enumerating ≥ 10 MPN *Salmonella*/100 ml, a 20% chance of enumerating ≥ 15 MPN *Salmonella*/100 ml, a 17% chance of enumerating ≥ 20 MPN *Salmonella*/100 ml, and a 1% chance of enumerating ≥ 60 MPN *Salmonella*/100 ml. All of the models are statistically significant at a P value of <0.05 , with the exception of the ≥ 3 -MPN *Salmonella*/100-ml model ($P = 0.815$).

DISCUSSION

Salmonella is present in Central Florida surface waters throughout the year. Surveys of surface waters for *Salmonella* in the Southeastern United States have reported similar findings; in North Carolina, surface water had a *Salmonella* prevalence of 54.7% (94/172) in 25-ml water samples (25), and in a southern Georgia watershed, 79.2% of water samples were *Salmonella* positive using a five-replicate, three-dilution (100, 10, and 1 ml) MPN method (26). A survey of the Suwannee River in northern Florida reported that 96% (106/110) of water samples were *Salmonella* positive using a three-replicate, five-dilution (500, 100, 50, 10, and 1 ml) MPN method (27). These findings are similar to those found by other researchers performing surface water samplings over extended periods of time at the same set of sites in more-geographically diverse locations. Two rivers and one creek, all in one Southern Ontario, Canada, water shed, had 78.4% (91/116) *Salmonella*-positive sam-

ples when locations were sampled twice monthly for 2.5 years (28). Other studies have reported lower *Salmonella* prevalence in surface waters. Surface waters in the Salinas Valley of California, surveyed using either Moore swabs or a 100-ml sample, report 7.1% (18/252) *Salmonella*-positive samples (29). The prevalence of *Salmonella* in the coastal waters of southern Morocco during a 4-year monthly sampling of six sites was 4.1% (10/243) (30). Similarly, *Salmonella* was present in 11% (16/145) of 100-ml samples of surface waters collected from fruit and vegetable farms in New York state (31). The presence of *Salmonella* throughout the year in Central Florida surface waters is similar to that in surface water surveys done throughout the Southeastern United States, especially when considering the increased sample volume and low limit of detection used in the current study.

No regulatory limit currently exists for *Salmonella* in agricultural waters, and as larger volumes of waters are screened for *Salmonella*, the limit of detection decreases accordingly (21). Protocols for *Salmonella* detection are expensive and time-consuming compared to standard water testing of 100 ml for generic *E. coli*. Mitigation strategies suited for treating typical volumes of water used in agricultural production when *Salmonella* might be detected are very limited. Therefore, the ability of index organisms, such as *E. coli*, to predict the probability of enumerating *Salmonella* levels in surface waters intended for agricultural use is desirable.

None of the index microorganisms measured strongly linearly correlated with the prevalence of *Salmonella* in the same Central Florida surface water sample. Many of the coliform log MPN/100 ml counts were at or above the upper limit of detection; this may be responsible for the lack of correlation between *Salmonella* and coliform populations. In the cases in which coliform populations were at or above the upper limit of detection, the population could have been higher and would not be appropriately reflected by the data point. All water samples in this study with coliform counts above 3.2 log MPN/100 ml, the upper limit of detection, were recorded as ≥ 3.2 log MPN/100 ml, adding error into the calculations of the correlation coefficients.

Numerous publications have described the lack of correlation or presence of weak correlations between index microorganisms and pathogens in surface water (7, 32–36). Moderate correlations were obtained when *E. coli* or coliform data from the current study were analyzed for correlations with *Salmonella* populations for individual sites (Table 2); however, when the data were aggregated for all site correlations between *E. coli* or coliforms and *Salmonella*, both had R^2 values of <0.1 , indicating that other factors beyond those studied here influence the relationships between pathogens and index microorganisms. Studies that report similar results conclude that high correlations between pathogens and index microorganisms may be temporal, random, site specific, or time specific (35). High correlations between pathogens and index microorganisms often occur at point sources, such as surface waters impacted by improperly treated sewage or runoffs from livestock farms, where the levels of index microorganisms and pathogens are consistently high for a longer period of time (35). Our study was conducted in rural agricultural Florida, where the influence of raw sewage effluent is not expected and was not observed. The occurrence of *Salmonella* in the environment may be at a much higher frequency than previously assumed; under some conditions, such as those in Central Florida, *Salmonella* may not be transient in nature within surface waters as was previously re-

ported (37). In tropical and subtropical watersheds, *E. coli* may be autochthonous rather than an indication of fecal contamination (38, 39). Results reported here, along with those of other studies carried out in geographically similar locations (25–27), suggest that the presence of *Salmonella* in the environment may not result solely from a recent fecal contamination. Correlations between *E. coli* and *Salmonella* populations in Central Florida surface waters may not be explicit evidence of fecal contamination but rather of conditions favorable to the survival of both organisms.

Not only did our analysis find no strong correlations between *E. coli* or coliform populations and *Salmonella*, possibly due to no influence of improperly treated sewage or runoff from livestock, *Salmonella* levels were not correlated with rainfall or seasons as has previously been reported (7). In our analysis, average rainfall, whether aggregated for 24 h, 1 week, or 1 month (prior to sampling), did not correlate with *Salmonella*, *E. coli*, or coliform levels. Conflicting results have been reported in several other studies that also attempted to determine a correlation between rainfall and pathogen prevalence. A southern Ontario, Canada, surface water study found that the lowest occurrence of *Salmonella* did coincide with the month (February) with the lowest recorded precipitation, air temperature, and water temperature (28). A positive correlation between *Salmonella* prevalence and rainfall (mm/day) was reported, without actual R^2 values being present, during a study in southern Morocco (30). The Morocco study analyzed 100 ml of seawater collected monthly at six sites for a 4-year duration. In New York state, *Salmonella* had a higher prevalence in surface waters when measurable precipitation occurred within 3 days prior to sampling; however, this was observed only in areas of poorly drained soils (31). The differing results may suggest that rainfall does have some effect on *Salmonella* prevalence in surface waters, not as a direct correlation, but perhaps in a multifactorial way, including more characteristics than those noted in the current study.

A study in Puerto Rico attempted to correlate rainfall from 24 h, 48 h, and 1 week prior to water sampling for fecal coliforms and found no correlation between fecal coliforms and precipitation in any of the 10 sampling sites (40). Santiago-Rodriguez et al. (40) suggested that rainfall may cause correlations to increase due to resuspension of fecal coliforms from sediments but that rainfall may have a possible dilution effect on fecal coliform concentrations, which may lower correlations (40). Some studies have indicated that turbulence caused by rainfall may resuspend pathogens or indicator microorganisms from sediments, raising their levels in the waters by 100- or 1,000-fold and that the depth and size of the water body dampen any rainfall dilution effect on the indicator/pathogen levels (7). Our study did not find a correlation between rainfall and *Salmonella* or *E. coli* and coliform populations. At no point during the water sampling procedure was the sediment disturbed; all sampling sites had great enough depth, and care was taken not to disturb the sediment during sampling. The depth of water varied at and between each of the sampling locations. The consistently low turbidity (Fig. 4) evidences the lack of sediment disturbance. Higher sample turbidity did not correlate with larger *Salmonella* populations. This may indicate that overt sediment disturbance is not a major contributing factor to the presence of *Salmonella* in Central Florida surface waters.

Correlations were not influenced by season, possibly due to the relatively small temperature changes in Central Florida's subtropical climate. However, in Southern Ontario and New

York state, areas with much larger seasonal temperature variations, *Salmonella*-positive samples were not significantly different between seasons (28, 31). The rise and fall in the levels of biological characteristics was random and did not coincide with increasing temperatures in the summer and reduced temperatures in the winter. This is contrary to the finding of Gorski et al. (29), who noted a noticeable seasonal trend in the prevalence of *Salmonella* in surface water samples from the Salinas Valley of California (29). Further work is required to acquire a better understanding of whether, and over what range, water temperature would have an effect on *Salmonella* prevalence and whether geographic location or other climatic factors also have an effect.

Physicochemical water characteristics have been used to monitor the chemical and microbiological qualities of drinking and recreational water. Data from some sites (Table 2) showed that turbidity, ORP, and conductivity can predict the levels of *Salmonella* in surface water, but when aggregated across all sites, none of these parameters produced acceptable correlations. Although R^2 values were low, indicating the possible impact of additional unmeasured factors, such as dissolved oxygen, total assimilable carbon, or biological oxygen demand, Table 3 describes interesting and significant ($P < 0.05$) relationships between the measured parameters and the microbial concentration. For instance, when *E. coli* or solar radiation levels increase, these increases are correlated with increases in *Salmonella* levels. The positive correlation of solar radiation with *Salmonella* may be partially explained by *Salmonella* being more resistant to solar radiation than are other bacterial pathogens, such as *E. coli*, *Shigella flexneri*, and *Vibrio cholerae* (41). As solar radiation increases, less-resistant bacteria are inactivated, leaving fewer bacteria to compete for nutrients; *Salmonella* would have access to a greater amount of nutrients as solar radiation increases.

When conductivity levels increase, there is a highly statistically significant ($P < 0.0001$) inverse correlation with the *E. coli* concentration. Conductivity of surface water is an indirect measure of salinity and total dissolved solids and may be affected by storm water and runoff (42). An inverse correlation between indicator bacteria (*E. coli*, enterococci, and *Bacteroides*) and conductivity or salinity was reported for estuary waters in eastern North Carolina (42). The inverse correlation noted in the current study may be due to storm water and runoff effects in which the addition of the storm water or runoff in carrying salinity or other total dissolved solids from the surrounding area also dilutes the *E. coli* concentration. The sampling sites used in the current study were not influenced by seawater. Though these relationships are statistically significant, low coefficient of correlation (<1 or <-1) values indicate that other as-yet-unknown factors are also involved.

The complex nature of the index or indicator and pathogen relationship makes predicting the levels of pathogens through index/indicator microorganisms challenging; simple, linear relationships cannot be relied upon for predicting pathogen levels from indicator populations. Logistic regression analysis can be used where a linear relationship between variables is weak. Logistic regressions provide a measure of predictability and have been used to predict the efficiency of a water treatment process in which the pathogen of interest is poorly correlated to its index/indicator microorganisms (35). *E. coli* concentration has a fair ability to predict the *Salmonella* concentration (Fig. 5) in surface water in Central Florida. The model is limited to predictions with the range

of *E. coli* concentrations used to develop the model, ranging from the limit of detection ($-1 \log$ MPN/100 ml) to the maximum possible enumeration level for *E. coli* ($3.2 \log$ MPN/100 ml). Predictive models (linear) for *E. coli* have been reported for individual sites for inland recreational lakes in Ohio; explanatory variables included rainfall, turbidity, wind, and water temperature (43). Some site models were validated by data collected from an independent year, while others were not able to be validated due to climatic changes (43). In the same study, no predictive models were generated for *Salmonella*, but the authors did note that water samples that were PCR positive for *Salmonella* had higher median concentrations of *E. coli* (43). The performance of our *Salmonella* predictive model was able to be evaluated by data collected during an independent year or from independent locations.

As part of the implementation of the Food Safety Modernization Act (FSMA), the U.S. FDA has proposed produce safety rules for safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption. The proposed produce safety rule recommends collecting 5 samples. Those samples should have a mean *E. coli* concentration of ≤ 126 MPN/100 ml, and no single sample should exceed 235 MPN generic *E. coli*/100 ml (6). Based on the model for surface water in Central Florida, 126-MPN/100-ml and 235-CFU/ml criteria translate to probabilities of containing ≥ 5 MPN *Salmonella*/100 ml of 54.4% and 56.2%, respectively. In addition, the levels of generic *E. coli* in our surface water samples exceeded the recommended criteria ca. 30% of the time, suggesting that, according to proposed standards, some sites tested may be unsuitable for agricultural use.

The absence of strong correlations (low R^2) between index/indicator microorganisms or chemical or physical indicators and *Salmonella* levels limits their ability to predict the prevalence of *Salmonella*. However, significant positive relationships occur between *E. coli* and *Salmonella* levels and between solar radiation and *Salmonella* levels. These may offer a fast but qualitative indication of the degree of *Salmonella* risk. Logistic regression analysis appears to provide the best alternative to a weak quantitative linear model. Thus, pending the discovery of new, more-reliable index/indicator microorganisms, *E. coli* levels can be used to predict the probability of enumerating a standard *Salmonella* level in Central Florida surface water and provide a preliminary measure of risk. Given the dynamic, heterogenous, and complex nature of surface water ecosystems, the challenge remains to find an indicator parameter that is easy, rapid, and inexpensive to test for and that has a more intimate relationship, beyond causal association, with the pathogen and is therefore less sensitive to extraneous factors.

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