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Comparative Assessment of the Microbial Quality of Agricultural Water on Kansas and Missouri Fresh Produce Farms

ABSTRACT

In 2015, the U.S. States Food and Drug Administration published the Produce Safety Rule (PSR), providing guidance for growers to minimize food safety risks associated with growing, harvesting, packing, and holding fresh produce. To mitigate foodborne outbreaks attributed to contaminated agricultural water, the PSR requires growers to test their water for microbial contamination. The increased production of fruits and vegetables in Kansas and Missouri necessitates the investigation of agricultural water quality in these states. This study assessed and compared the prevalence of generic *Escherichia coli* in agricultural water sources in both states. A total of 426 agricultural water samples were analyzed using the IDEXX Colilert with Quanti-Tray/2000 method. Although there were no statistically significant differences in the prevalence of *E. coli* in agricultural waters detected between the two states ($P < 0.4023$), the average number of *E. coli* in surface water sources (158.7 most probable number [MPN]/100 mL, $n = 247$) was statistically greater than that of groundwater sources (20.4 MPN/100 mL, $n = 179$, $P < 0.0001$), and seasonal effects were detected ($P < 0.0001$). These results demonstrate the higher microbial risk of surface water compared with groundwater in both states and the need for continued grower education on safe water management practices.

INTRODUCTION

Fresh produce is a major vehicle for foodborne pathogens *(11)*, because such food commodities are often consumed raw and there is no kill step *(30, 34)*. From 2009 to 2018, there were 753 foodborne outbreaks associated with leafy greens alone in the United States, resulting in 15,603 illnesses, 1,604 hospitalizations, and 151 deaths *(4)*. In 1998, the U.S. Food and Drug Administration (FDA) published a general guide of voluntary on-farm practices to minimize microbial safety hazards associated with fresh produce *(36)*. However, following numerous foodborne outbreaks attributed to contaminated agricultural water in commodities such as leafy greens *(20, 27)*, tomatoes *(1)*, and melons *(42)*, water management practices became a critical theme in produce

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safety *(21)*. In 2015, the FDA finalized the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR), outlining science-based practices to reduce the prevalence and transmission of pathogens to produce, including through agricultural water *(9)*.

The FSMA PSR highlights information on contamination routes and best practices to minimize risks of contamination. One of the routes focuses on agricultural water quality and highlights the needs for testing water and establishing a microbial water-quality profile (MWQP) *(38)*. This profile is developed using generic *Escherichia coli* as an indicator organism for fecal contamination, with a testing frequency dependent on the inherent risks associated with the agricultural water source *(38)*. There are three primary water source types associated with produce operations: public water supplies, groundwater, and surface water. Public (municipal) water supplies assume the lowest risk of microbial contamination, because the water is treated and its microbial quality is regularly monitored by a utility entity; however, this source may be cost prohibitive for large operations that consume a great amount of water *(45)*. Groundwater (e.g., well water) is considered a moderate risk with the potential for microbial contamination events because of poor aquifer quality and/or well structural integrity. Although more convenient to access and use *(12)*, surface water (e.g., ponds, rivers, creeks, and rainwater catchment systems) are considered the highest risk, because growers are generally unable to completely isolate surface water sources from external sources of microbial pollution (i.e., contaminated soil and wild animal and/or livestock feces). Besides the water source, factors such as proximal livestock density *(15, 19)*, climate *(22, 28)*, and frequency of water treatments (e.g., chlorine shock and filtration) *(3, 40)* can affect microbial water quality.

The PSR states that all agricultural water must be "safe and of adequate sanitary quality for its intended use" (21 CFR 112.43) *(38)*. Based on a rolling 4-year dataset of *E. coli* test results, an agricultural water source used for preharvest operations (i.e., irrigation and fertigation) must have a geometric mean (GM) of less than 126 CFU of *E. coli* per 100 mL of water and a statistical threshold value (STV) of 410 CFU or less of generic *E. coli* per 100 mL of water. Both mathematical values are important when building a MWQP, because the GM provides information on the average amount of generic *E. coli* in the water source and the STV captures the variation in *E. coli* levels during the year—potentially as a result of adverse events (i.e., rainfall). Water sources used for postharvest purposes (i.e., rinsing produce or washing hands) must contain no detectable *E. coli* per 100 mL of water *(38)*. Moreover, untreated surface water is not permitted for postharvest use. Although the FDA extended the compliance dates of the PSR agricultural water testing requirements to facilitate grower adherence to the rule, studies indicate that agricultural water remains one of the least understood topics of the FSMA PSR in midwestern states *(5, 25, 26, 29)*. To the authors' knowledge, the microbial safety of agricultural waters in these states has rarely been studied, with the exception of Iowa *(2)*. Bhullar et al. *(2)* reported few contamination events for groundwater sources in Iowa; however, contamination of agricultural surface waters with generic *E. coli* was prevalent in Iowa, with some surface water samples exceeding the FDA's maximum allowed GM thresholds.

Each year, Kansas and Missouri growers produce approximately $26 million *(17)* and $81 million (6) of fruits and vegetables, respectively, with recent notable increases in the production of specialty crops. For example, berry production increased dramatically in Kansas, with the number of blueberry farms increasing by 269%, blueberry acres increasing by 250%, blackberry farms increasing by 112%, and blackberry acres increasing by 260% from 2007 to 2017 *(23)*. Thenumber of Kansas farms producing tree fruit also increased by 46% from 2007 to 2017 *(23)*. To support the outputs of this growing industry, the purpose of this study is to understand the microbial quality of agricultural waters used by growers in Kansas and Missouri and identify opportunities for extension education and outreach. The objectives of this study are thus to (1) evaluate and (2) compare the prevalence of microbial contamination in agricultural water sources on Kansas and Missouri farms.

MATERIALS AND METHODS

Sample collection and submission

From 2018 to 2020, individual growers, extension educators, or trained laboratory personnel collected water samples from agricultural water sources on Kansas and Missouri produce-growing operations *(Figure 1)*, following shared instructions prepared by the Kansas State University (KSU)/University of Missouri Extension produce safety team (see *supplemental documentation*). The water samples were collected in 100-mL sample containers with added sodium thiosulfate (IDEXX Laboratories, Westbrook, ME), to reduce the effect of residual chlorine on *E. coli* stability during transit, and then mailed overnight or submitted in person in a refrigerated cooler box with ice to a microbial water-quality testing laboratory for analysis according to their state of residence. Kansas growers submitted water samples to the Food Safety Laboratory at KSU in either Olathe or Manhattan, whereas Missouri growers submitted the samples to the KSU lab in Olathe or the Missouri State Public Health Laboratory (MSPHL). The samples were accompanied by a submission form specific to the laboratory to provide information on water sources (see *supplemental documentation*). Water samples were received from various locations in eastern Kansas and throughout Missouri, as shown in *Figure 1*. Because these labs provide microbial water testing services free of charge to produce growers through grant funding, multiple entries from the same grower may have occurred throughout each year. In addition, it was unknown whether the operations that submitted samples for analysis treated their agricultural water before use in production or postharvest activities.

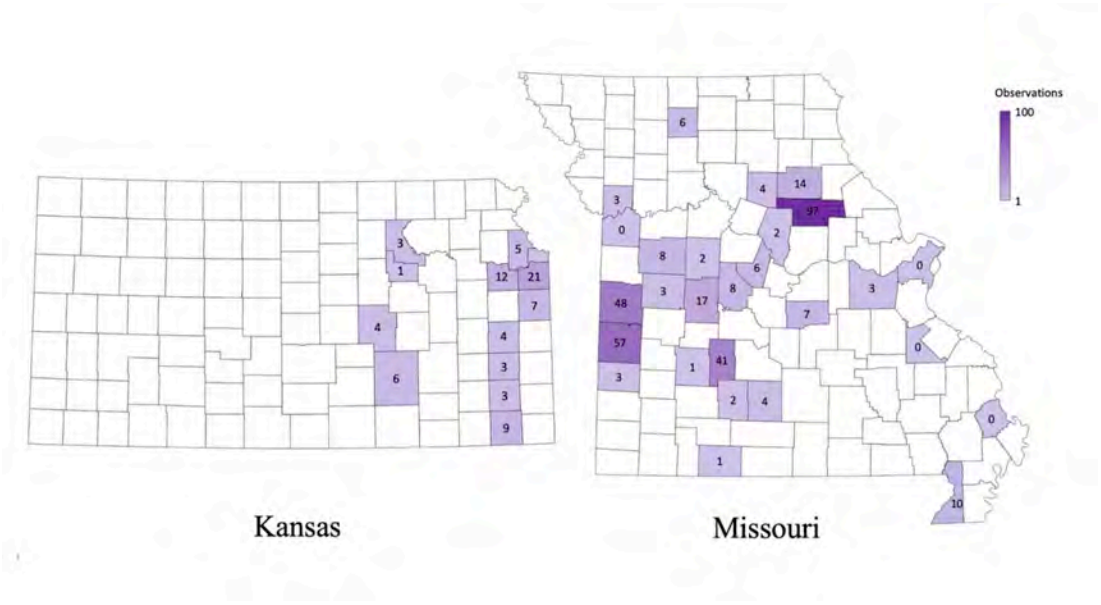

Figure 1. Distribution of agricultural water source samples from counties in Kansas and Missouri.[a]

[a]The number in each county indicates the number of samples included in the dataset from that county.

Sample testing methodology

The three labs used the IDEXX Colilert with Quanti-Tray/2000 test method *(37)* (IDEXX Laboratories, Westbrook, ME) to obtain the most probable number (MPN) of generic *E. coli* per 100 mL of water. One snap-pack of Colilert reagent was added to each 100-mL water sample and dissolved through vigorous shaking before being sealed in a Quanti-Tray and incubated for 24 h at 35°C ± 0.5°C. The Colilert reagent is a proprietary Defined Substrate Technology wherein two nutrient indicators—ortho-nitrophenyl-β-galactoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG)—act as the major source of carbon for coliforms and *E. coli* (respectively), promoting their selective growth in the sample during incubation *(10)*. β-glucuronidase, an enzyme conserved in 94 to 96% of *E. coli (13)*, metabolizes MUG, cleaving the MUG-bound fluorescent reporter 4-methyl-umbelliferone. The MPN of generic *E. coli* was determined based on the number of large and small wells, which fluoresced under ultraviolet light (Spectroline, Melville, NY); an MPN table was provided by the manufacturer (IDEXX Laboratories, Westbrook, ME) for the MPN calculations. The lower limit of detection was <1.0 MPN/100 mL, and the upper limit of detection was >2,419.6 MPN/100 mL. The FDA determined that this method was scientifically valid and at least equivalent in accuracy, precision, and sensitivity to the conventional U.S. Environmental Protection Agency (EPA) 1603 method for quantifying generic *E. coli* in water, which uses the CFU per 100-mL unit *(37)*.

Data preparation

The data from the KSU labs and the MSPHL were shared with the project team and reviewed before analysis. To prepare the dataset for analysis, the sample source was first classified as groundwater or surface water according to the sample description. Entries in which the samples exceeded a 24-h holding time were not used in subsequent analyses. Personal identifiers (e.g., grower name and address) were deleted from the working datasets to protect the privacy of the grower.

Statistical analysis

The study was considered an incomplete block design, with STATE as a fixed blocking factor. All generic *E. coli* counts were documented as MPN per 100 mL values and transformed to fit a logarithmic distribution for statistical analysis. For samples that exceeded the microbial testing threshold value (>2,419.6 MPN/100 mL), 2,419.6 MPN/100 mL was used for further analyses; 1 MPN/100 mL was used for values below the testing threshold value (<1 MPN/100 mL). To investigate the variation in generic *E. coli* concentrations attributed to season, the seasons were divided into winter (December to February), spring (March to May), summer (June to August), and fall (September to November). Differences between the means were computed using the GLIMMIX procedure (SAS 9.4, SAS Institute, Cary, NC) with the Tukey-Kramer adjustment for multiple comparisons of unequal sample sizes. Statistical significance was established at $P < 0.05$.

RESULTS AND DISCUSSION

Source type distribution

Previous literature has primarily emphasized the study and surveyance of surface water microbial quality *(18, 31, 33)*; the microbial quality of other types of agricultural water sources (ground, municipal, etc.) within a growing region has rarely been directly compared. The findings of this

TABLE 1. Source type distribution of water samples tested by source in Kansas and Missouri

Source Type	Kansas		Missouri	
	No. (n = 79)[a]	%	No. (n = 347)[a]	%
Groundwater	33	41.8	146	42.1
Surface water	46	58.2	201	57.9

[a]Number of groundwater and surface water samples submitted from produce growers in Kansas and Missouri in this study.

TABLE 2. Generic *E. coli* prevalence data in groundwater and surface water sources in Kansas and Missouri[a]

MPN/100 mL	Groundwater		Surface water	
	No. (n = 179)	%	No. (n = 247)	%
<1	145	81.0	55	22.3
1–126	31	17.3	152	61.5
126–2,419.6	2	1.1	35	14.2
>2,419.6	1	0.6	5	2.0

[a]Relative prevalence of generic *E. coli* (CFU/100 mL) in groundwater and surface water sources based on the provided data (N = 426).

study illustrate the considerable use of groundwater sources within produce-growing operations in midwestern states. In fact, 41.8% (33/79) of the water samples from Kansas and 42.1% (146/347) of the water samples from Missouri were from groundwater sources *(Table 1)*. Bhullar et al. *(2)* reported similar findings in Iowa, wherein 67% (69/101) of the agricultural water samples for microbial testing were collected from groundwater sources. Future studies would benefit from examining the microbial quality of agricultural water source types according to their usage in the region (preharvest or postharvest use) to facilitate more directed agricultural extension and outreach activities. In the Midwest and regions of similar groundwater usage trends, this directive would include investigating the microbial water quality of groundwater sources.

Prevalence of generic *E. coli* in agricultural water sources

Generic *E. coli* was detected in 77.7% (192/247) of the surface water samples and 29% (34/179) of the groundwater samples collected in this study *(Table 2)*. The widespread presence of generic *E. coli* in surface water sources is well documented, particularly because this source type is exposed to contamination events from contact with domestic or wild animal fecal matter *(39)*. Previous studies of surface water microbial quality from the midwestern states of Iowa *(2)* and Ohio *(44)* reported the presence of generic *E. coli* in 31% (32/101) and 96.9% (219/226) of the samples collected, respectively. Moreover, in Iowa, Bhullar et al. *(2)* noted 5.8% (4/69) of the groundwater samples contained generic *E. coli*; many of these groundwater samples were collected from shallow wells (<60 ft [<18.3 m]). Other studies have also shown *E. coli* contamination of groundwater sources *(8, 14, 16, 24)*, with one study *(8)* indicating that such contamination can be exacerbated by certain types of well construction. Further investigation is needed to determine the cause of the *E. coli* contamination of groundwater sources in Kansas and Missouri and ways to mitigate these risks.

Overall, the agricultural water source type was a significant source of variation in the concentration of generic *E. coli* (P < 0.0001), although there were no statistically significant differences detected between the states (P < 0.4023). This study not only reported higher microbial risk in surface waters but also concurs with the PSR's classification of surface water as higher risk and groundwaters as lower risk for microbial contamination *(38)*. The average *E. coli* concentration for agricultural water samples tested in Kansas and Missouri was 158.7 MPN/100 mL (n = 247) for surface water and 20.4

TABLE 3. Average generic *E. coli* concentration (CFU/100 mL) of groundwater and surface water sources in Kansas and Missouri[a]

Source Type	Kansas	Missouri	Overall
	n = 79	*n* = 347	N = 426
Groundwater	2.8 (*n* = 56)	24.3 (*n* = 123)	20.4 (*n* = 179)
Surface water	31.4 (*n* = 29)	188.3 (*n* = 218)	158.7 (*n* = 247)

[a]Average generic *E. coli* concentration (CFU/100 mL) in groundwater and surface water sources based on the provided data.

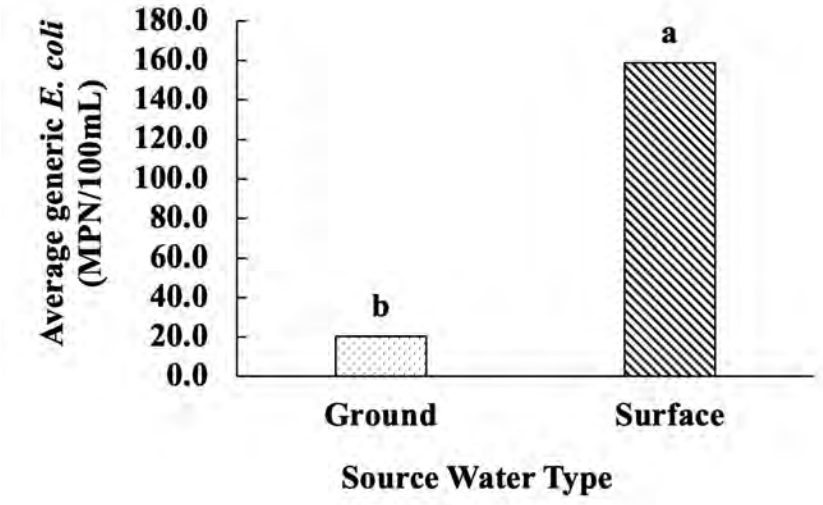

Figure 2. Average *E. coli* concentration for agricultural water sources in Kansas and Missouri.[a]

[a]Average *E. coli* concentration from groundwater and surface water sources used by produce growers in Kansas and Missouri (*n* = 426). Statistically significant differences between the log-transformed means of the source water types are denoted with letters (α = 0.05).

MPN/100 mL (*n* = 179) for groundwater source samples *(Table 3)*. Accordingly, the concentration of generic *E. coli* in surface water sources was significantly greater than that of groundwater sources ($P < 0.0001$).

Microbial risk of Kansas and Missouri source waters in relation to the PSR

Per the regulations outlined in the PSR, the results of this study indicate that surface waters in Kansas and Missouri are more likely to be unfit for use in production-related activities than groundwater sources *(Fig. 2)*. However, growers need to develop a water-quality profile to determine compliance with the PSR rule. More information on MWQP can be found in subpart E of the PSR.

The seasonality of generic *E. coli* concentrations in surface water sources are well recorded in the literature, with the concentration of generic *E. coli* typically increasing with rising air temperatures *(32, 43)*. In this study, season was a statistically significant source of variation ($P < 0.0001$). From further analysis performed post hoc, surface water sources contained a statistically higher concentration of generic *E. coli* than groundwater sources in the spring ($P < 0.0001$) and summer ($P < 0.0001$); no statistically significant differences were detected between the sources during the fall ($P = 0.0541$) or winter ($P < 0.2100$). The 2.0% (*n* = 5) of surface water samples and 0.6% (*n* = 1) of groundwater samples that exceeded the threshold value of the testing method (>2,419.6 MPN/100 mL) were recorded during the spring and summer months (April to August).

Potential challenges to PSR compliance

The findings of this study indicate that some produce growers in Kansas and Missouri may face barriers to accessing microbial water-quality testing services despite lowered costs. Many samples submitted could not be tested because they exceeded the 24-h hold time. The National Water Summit

facilitated by the Produce Safety Alliance in 2018 emphasized the cost of agricultural water testing as a major challenge to growers. During the summit, groups expressed concern that the estimate of $1,058 in testing costs per year per farm calculated by the FDA may not cover additional costs to the grower, such as time to collect and transport the sample or testing costs if the operation uses water from multiple sources *(41)*. Offsetting the cost of agricultural water testing for Kansas and Missouri growers was a critical motivator in requesting this grant funding, particularly as a study of food safety practices by Perry et al. *(25)* in the north-central region (encompassing Kansas and Missouri) revealed that up to 43% of the surveyed growers (n = 253) may not be testing their agricultural water. However, this study suggests sample transit and holding times as potential obstacles for growers in the Midwest, in addition to the cost. The database originally contained 760 observations, but only 507 (66.71%) observations could be used for this analysis. The remaining samples were excluded from the statistical analysis, because they exceeded the 24-h time limit for sample transit and holding specified by the EPA Revised Total Coliform Rule to prevent microbial decline *(2, 35)*. Potential actions to address these challenges are included in the "Conclusions and Recommendations" section of this manuscript.

Study limitations

Accessing the three laboratories' databases presented an excellent way to leverage existing data and capture a snapshot of regional water quality, but this methodology inherently carries a few limitations. First, the sampling region in Kansas is heavily east biased, whereas the sampling region in Missouri is more evenly distributed. Although the geographical distribution is likely a result of the sampling sites' proximity to the testing labs, this aspect introduces regional bias to the dataset. Furthermore, there are a larger number of produce growers in eastern Kansas than in western Kansas, based on the contacts that the project team receives from both parts of the state and membership in organizations such as the Kansas Specialty Crop Growers Association that is more heavily based in eastern Kansas. Effectively, the findings of this study may not reflect the reality of agricultural water quality used for produce in western Kansas, and further investigation to expand the dataset and include more sampling sites in western Kansas is needed. The databases also did not always specify from which source the samples were taken on each farm (i.e., pond A or pond B), so it was impossible to ascertain whether there were resamples or each data entry was a unique water source. As a consequence, only the average was reported in this manuscript, because it would be inappropriate to calculate the GM and STV for different water sources.

Conclusions and recommendations

To the authors' knowledge, this is the first study in Kansas and Missouri to provide an individualized and comparative analysis of the microbial quality of agricultural water sources used in produce-growing operations. Agricultural water regulations and management practices outlined in the FSMA PSR are among the least understood topics by growers in midwestern states *(5, 25, 26, 29)*. As the production of fresh fruits and vegetables increases in Kansas and Missouri, ensuring produce safety is critical for consumer health and for supporting the local and statewide economy. This study was designed to determine the prevalence of *E. coli* in agricultural water sources from Kansas and Missouri produce-growing operations. The study findings highlighting the contamination of surface water and groundwater with generic *E. coli* reinforce the need for effective, accessible water-quality control and treatment methods to minimize the microbial risks on fresh produce farms.

The results of this study largely coincide with the conclusions of previous studies in the Midwest *(2, 5, 25, 26)*: that continued extension education and outreach are needed to improve grower knowledge on water testing requirements and treatment methods. Kansas State Research and Extension and University of Missouri Extension personnel are fulfilling this need by providing digital materials for growers regarding produce safety and water safety at https://www.ksre.k-state.edu/foodsafety/produce/index.html and https://extension.missouri.edu/programs/food-safety, respectively. Continued efforts are also being made to provide printed materials for Plain community growers (Amish and Mennonite) as knowledge gaps become increasingly clarified *(7, 25)*.

Agricultural water treatment is another factor affecting microbial risk that was not explored in this study. Identifying the common chemical and/or physical agricultural water treatments (if used) within Kansas and Missouri produce-growing operations will aid in guiding extension education on agricultural water treatment methods and may help improve produce safety. As a recommendation for future agricultural water surveys, it would be beneficial to continue to investigate the microbial quality of varying agricultural water source types (as opposed to solely surface water) and information on environmental factors contributing to an increased *E. coli* prevalence (i.e., temperature, precipitation, animal grazing, farming and practices) to establish a water-quality profile reflective of the sources commonly used in the region and to facilitate more directed produce safety extension and outreach activities. These approaches will help with drawing science-based conclusions on minimizing food safety risks.

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