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# Differential Survival of Non-O157 Shiga Toxigenic *Escherichia coli* in Simulated Cattle Feedlot Runoff

Lisa M. Durso,<sup>1</sup> John E. Gilley, and Daniel N. Miller

## Abstract

Environmental survival time is important when evaluating adverse health outcomes from foodborne pathogens. Although outbreaks associated with manure-impacted irrigation or runoff water are relatively infrequent, their broad scope, regulatory importance, and severe health outcomes highlight the need to better understand the environmental survival of manure-borne pathogens. Shiga toxigenic *Escherichia coli* (STEC) are excreted in feces and persist in the environment until they die or recolonize a new host. Surface waters contaminated with manure-borne STEC can infect humans through drinking and recreational water use or irrigated crops that are minimally cooked. In this study, manure-impacted water microcosms mimicking beef cattle feedlot runoff were used to assess survival of STEC strains representing seven STEC serotypes (O26, O45, O103, O111, O121, O145, and O157) and persistence of target O antigen genes. Microcosms were sampled over the course of 1 year, and the entire experiment was repeated in a second year. Culture and polymerase chain reaction (PCR)-based techniques were used for detection and enumeration. Serotype-specific survival results were observed. Both STEC O26 and O45 declined slowly and remained culturable at 24 months. In contrast, STEC O121 and O145 decreased rapidly (−0.84 and −1.99 log<sub>10</sub> abundance per month, respectively) and were unculturable by months 4 and 5, but detectable by PCR for a mean of 4.5 and 8.3 months, respectively. STEC O103, O111, and O157 remained culturable for a mean of 11.6, 5.5, and 15 months and detectable by PCR for a mean of 12, 13.8, and 18.6 months after inoculation, respectively. Results document that some STEC serotypes have the biological potential to survive in manure-impacted waters for extended periods of time when competing microflora are eliminated. Serotype-specific differences in survival of target bacteria and persistence of target genes were observed in this sample set, with STEC O26 and O45 strains appearing the most robust in these microcosm studies.

**Keywords:** Shiga toxigenic *E. coli*, STEC O157, non-O157 STEC, survival, environment, manure

## Introduction

**S**HIGA TOXIGENIC *ESCHERICHIA COLI* (STEC) are foodborne pathogens associated with potentially life-threatening gastrointestinal illness. Severe complications such as hemolytic uremic syndrome or death are associated with outbreaks of this group of pathogens (Fremaux *et al.*, 2008). STEC with the O157 serotype emerged widely as an important foodborne pathogen in the 1980s, associated primarily with beef (Kaper and O'Brien, 2014). In 1994, STEC O157 was declared an adulterant in beef in the United States, and in 2012, six additional STEC serotypes (non-O157 STEC) were added: O26, O45, O103, O111, O121, and O145 (USDA, 2011). While early U.S. STEC outbreaks were pri-

marily associated with STEC O157 in cattle production systems, today, non-O157 STEC outbreak cases in the United States outnumber STEC O157 cases (CDC, 2016), and in 2018–2019, the CDC reported twice as many lettuce-associated versus beef-associated STEC outbreaks (<https://www.cdc.gov/ecoli/outbreaks.html>).

Manure-borne STEC is a major contaminant of leafy greens, with transmission occurring through direct contact with wildlife or cattle feces and through runoff, dust, and insects (Berry *et al.*, 2019a, 2019b). There is also growing concern with the transmission of STEC to produce through irrigation with STEC-contaminated water (Gelting *et al.*, 2011; Mikhail *et al.*, 2018; Haymaker *et al.*, 2019), including the potential for uptake of STEC from manure-impacted

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surface and irrigation water and internalization into plant tissue (Merget *et al.*, 2019). Specifically, animal manure-impacted irrigation water is considered under the Food Safety Modernization Act to be a potential primary source of contamination for produce (FDA, 2015), with produce-related events often resulting in multistate outbreaks (Heiman *et al.*, 2015). Although outbreaks associated with manure-impacted irrigation water are relatively infrequent, their broad scope, regulatory importance, and severe health outcomes highlight the need to better understand the environmental survival of these important pathogens.

*E. coli*, including STEC, naturally cycle between the warm, nutrient-rich, lower gastrointestinal tract (GIT) of animals, which is their primary habitat, and environments external to the host, including water, soil, and air (Savageau, 1983). Individual *E. coli* cells persist in the natural environment until they either recolonize a mammalian host or they die (Savageau, 1983). Thus, environmental survival time is a critical component contributing to contamination of produce and the ability of STEC to eventually reach and infect a human host (Brouwer *et al.*, 2019).

Increasing evidence reveals that there are important phenotypic differences in how STEC O157 and non-O157 STEC persist in the GIT, manure, food, and soils (Free *et al.*, 2012; Brouwer *et al.*, 2019; Merget *et al.*, 2020). For example, Ma *et al.* (2014) examined the persistence of STEC in agricultural soil microcosms and found that some non-O157 STEC serotypes survived significantly longer than STEC O157s, citing strain and soil type as drivers for the observed differences. There is a substantial body of literature examining the survival of STEC O157 under various extraintestinal conditions (Fremaux *et al.*, 2008; Topalcengiz and Danyluk, 2019; Reynolds *et al.*, 2020). However, data characterizing the survival potential of STEC and non-O157 STEC in runoff-contaminated irrigation water are sparse and inconsistent. The goal of the present investigation was to assess the biological potential of individual STEC strains to persist in manure-impacted water microcosms. We hypothesize that STEC will display a variety of serotype-specific survival phenotypes. If true, then extrapolating specific O157 survival characteristics to the broader *E. coli* community may not be supported.

## Materials and Methods

### Simulated runoff

Soil was collected from the University of Nebraska Rogers Memorial Farm, which had been used in previous field runoff studies (Durso *et al.*, 2019). The Aksarben soil from which samples were collected developed in loess under prairie vegetation and is considered a benchmark soil for the Western Corn Belt. The site had been cropped to corn the previous cropping season. Soil was collected from the surface of selected locations (top 10 cm), brought into the laboratory, dried, and sieved. Beef cattle manure was collected from the U.S. Meat Animal Research Center feedlot near Clay Center, NE. An aliquot was sent to Ward Laboratories (Kearney, NE) for nutrient analysis to determine the agronomic rate for use in laboratory tests (Supplementary Table S1). Manure was added to dried soil at a rate of 25.8 kg P ha<sup>-1</sup> P to meet the 1-year P-based application requirement for an expected corn yield of 9.4 Mg ha<sup>-1</sup>. Five grams of manure-amended soil

was mixed into 787 mL of sterile distilled water in a 1-L sterile Nalgene bottle, resulting in turbidity of 600–700 nephelometric turbidity units, similar to field-collected runoff (Durso *et al.*, 2017). To determine the ability of each STEC serotype to survive or persist and to eliminate the stochasticity and variability introduced by competition from and predation by competing microflora, the microcosms were autoclaved before inoculation. Once cooled to room temperature, bottles were inoculated and stored at 4°C for the duration of the experiment to maximize bacterial survival time and to represent cooler temperatures outside of the GIT. Measurements were taken by removing bottles from the cooler, thoroughly mixing the bottles by shaking for 30 s, and taking aliquots for dilution and plating, after which the bottles were returned to storage.

### Bacterial strains and culture conditions

Seven STEC serotypes were used for this study: O26, O45, O103, O111, O121, O145, and O157 (Supplementary Table S2). To facilitate enumeration, strains were tagged with nalidixic acid resistance, as described previously (Ma *et al.*, 2014). STEC cultures were grown in tryptic soy broth containing 25 µg/mL nalidixic acid (TSB+Nal) overnight at 37°C. Cultures were adjusted to OD<sub>600</sub> = 0.300, equivalent to a concentration of ~10<sup>8</sup> colony-forming units (CFUs)/mL. Each culture was serially diluted and spread-plated on TSA+Nal in duplicate to confirm the exact concentration of the stock culture. Cultures were adjusted by concentration so that each simulated runoff container was spiked with 10<sup>6</sup> CFUs/mL. All experiments were performed using triplicate bottles for each serotype, for a total of 21 simulated runoff bottles (7 serotypes, 3 replicates per serotype), evaluated over the course of 1 year. The entire experimental design was replicated for a second year for four of the seven serotypes that had displayed either robust survival (O26 and O45) or quick decline (O121 and O145). For microcosms from year 1, when cells remained culturable or DNA persisted in triplicate samples after the initial 12-month study was completed, those bottles were evaluated monthly through year 2 to obtain 3-log<sub>10</sub> reduction times.

### Bacterial Enumeration

The simulated runoff in each prepared bottle was plated preinoculation and directly after being inoculated with ~10<sup>6</sup> CFUs/mL (Day 0). The overnight cultures used for inoculation were also immediately plated to determine the exact spike for each strain. Subsequent aliquots were plated, in duplicate, for enumeration in the simulated runoff on the following schedule: Day 1, Day 2, Day 5, Day 7, Day 14, Day 21, Day 28, Day 36, and Day 42 and then monthly until two consecutive plating instances produced zero colonies. Once quantification was no longer possible, aliquots were enriched for presence/absence detection.

At each sampling date, 1 mL of spiked simulated runoff water was diluted in 1X phosphate-buffered saline and then spread-plated, in duplicate, on tryptic soy agar +25 µg/mL nalidixic acid at the levels required to produce countable plates with 20–200 colonies per plate. Samples were thoroughly mixed by shaking for 30 s before aliquots were taken for dilution and plating. Plates were incubated at 37°C for 24 h for all samples, except those inoculated with STEC

O111. The STEC O111 plates were incubated at 37°C for 48 h for ease of counting due to observed slower growth. Following incubation, plates were counted and 10 colonies from each bottle were selected for confirmatory polymerase chain reaction (PCR). Confirmatory PCR primers and thermocycling conditions are listed in Table 1. All isolates from countable plates were confirmed with PCR once fewer than 20 isolates grew on the 10<sup>-1</sup> plates. For the 10<sup>0</sup> dilution plates with 1–10 colonies, all colonies were isolated and screened. Plating for enumeration was continued until two consecutive instances with zero colonies were observed (i.e., no colonies on the 10<sup>0</sup> plates).

When colonies were no longer observed after direct plating of the simulated runoff on either of the duplicate plates at the lowest dilution level, an enrichment detection method was utilized to determine if viable STEC could be enriched (culture based) or STEC genes (PCR based) could be detected. One milliliter of the spiked simulated runoff in the bottle was combined with 9 mL of TSB+Nal and enriched overnight at 37°C for 24 h (or 48 h for O111 for ease of counting due to slower growth), followed by plating for isolates and isolate screening using serotype-specific PCR (culture based). Additionally, the enriched sample was directly screened for serotype-specific genes (PCR based). This enrichment/PCR-based detection was performed on each sample bottle until negative PCR results were obtained for two consecutive sample dates, at which time the incubation was discontinued.

#### Statistical analysis

The various STEC strains were compared based upon changes in log<sub>10</sub> abundance during the simulated runoff incubation. Strains were assessed for a postinoculation

increase, differences in number of days for a 3-log<sub>10</sub> reduction, and for month of last detection using an analysis of variance (ANOVA) (proc general linear models [GLM]) and least squares means comparison using Student *t*-tests (SAS, version 9.2; SAS Institute, Cary, NC). Linear regression was first used to calculate the rate of log<sub>10</sub> abundance change (slope of the regression line) for each replicate using data from month 1 through month 12, followed by an ANOVA (proc GLM) and least squares means to determine differences (*p* < 0.05) between strains. Differences in survival times (using the number of months elapsed from the onset of the experiment until last detection by either culture-based or PCR-based methods) were determined using the least squares means. Since strains O45, O26, and O103 survived through the last month of the incubation, the total number of incubation months (30 or 20) was used for statistical purposes.

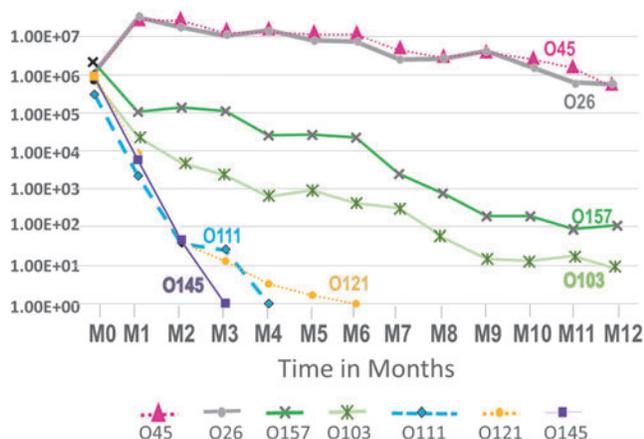
#### Results

Serotype-specific survival differences were observed as the abundance of STEC strains changed during the incubation (Fig. 1). Survival times for the seven STEC strains studied ranged from 3 to at least 30 months (Table 2). Three of the seven strains (O26, O45, and O103) initially increased in abundance (from ~10<sup>6</sup> to 10<sup>7</sup> CFUs/mL) within the first 2 weeks after inoculation into the manure-impacted water microcosms (Fig. 2) and remained culturable at the 12-month sampling point. In contrast, STEC O111, O121, and O145 strains decreased immediately upon inoculation into the simulated runoff and culturable populations declined below enumerable levels within 6 months (Fig. 1). O157 showed no increase in abundance, but was culturable for the full 12 months of the experiment.

TABLE 1. POLYMERASE CHAIN REACTION PRIMERS AND ANNEALING TEMPERATURES

STEC target	Primer sequences (5'-3')	Amplicon size (bp)	Thermocycling conditions	Citation
O26	AAATTAGAAGCGGTTTCATC CCCAGCAAGCCAATTATGACT	596	95°C for 15 min; 35 cycles at 94°C for 60 s, 56°C for 60 s, and 72°C for 60 s; and 1 cycle at 72°C for 10 min	Paton and Paton (1998)
O45	GGGCTGTCCAGACAGTTCAT TGACTGCACCAATGCACCT	890	94°C for 5 min; 35 cycles at 94°C for 30 s and 67°C for 80 s; and 1 cycle at 68°C for 7 min	Paddock <i>et al.</i> (2012)
O103	TAAGTACGGGGGTGCTTTTT AAGCTCCCGAGCACGTATAA	716	94°C for 5 min; 35 cycles at 94°C for 30 s and 67°C for 80 s; and 1 cycle at 68°C for 7 min	Paddock <i>et al.</i> (2012)
O111	AGAGAAATTATCAAGTTAGTTCC ATAGTTATGAACATCTTGTTTAGC	406	95°C for 15 min; 35 cycles at 94°C for 60 s, 53°C for 60 s, and 72°C for 60 s; and 1 cycle at 72°C for 10 min	Paton and Paton (1998)
O121	TCATTAGCGGTAGCGAAAGG TTCTGCATCACCAGTCCAGA	587	94°C for 5 min; 35 cycles at 94°C for 30 s and 67°C for 80 s; and 1 cycle at 68°C for 7 min	Paddock <i>et al.</i> (2012)
O145	TGCTCGACTTTTACCATCAAC AACCAACACCATAACACCTTGCTT	374	94°C for 5 min; 35 cycles at 94°C for 30 s and 67°C for 80 s; and 1 cycle at 68°C for 7 min	Paddock <i>et al.</i> (2012)
O157	CGGACATCCATGTGATATGG TTGCCTATGTACAGCTAATCC	259	95°C for 15 min; 35 cycles at 94°C for 60 s, 53°C for 60 s, and 72°C for 60 s; and 1 cycle at 72°C for 10 min	Paton and Paton (1998)

According to BLAST-NCBI, the polymerase chain reaction primers align with each target serotype. STEC, Shiga toxinigenic *Escherichia coli*.



**FIG. 1.** Enumeration of culturable cells over time. Plating for enumeration of multiple STEC serotypes was continued until there were 2 consecutive instances with 0 colonies on the 100 plates. Microcosms consisted of 5 g of manure-amended soil added to 787 mL of sterile distilled water, resulting in turbidity of 600–700 nephelometric turbidity units, similar to runoff collected from the field. Nomenclature: (M) denotes month; STEC strains are listed by somatic (O) antigen. STEC, Shiga toxicogenic *Escherichia coli*.

Comparing the 3- $\log_{10}$  reduction times showed that STEC strains varied considerably, ranging from 37 to 576 d, with statistically significant differences (Table 2). One group (O26 and O45) exhibited strong resilience (>500 d for a 3- $\log_{10}$  reduction) to conditions in the simulated runoff, while another group (O103, O111, O121, and O145) was much more sensitive to conditions (<50 d for a 3- $\log_{10}$  reduction). STEC O157 exhibited a more moderate response and displayed a 3- $\log_{10}$  reduction in 168 d.

Comparing month of last detection as a survival metric between STEC strains could provide insights into strain variation; however, it will likely be strongly influenced by the initial STEC abundance in the simulated runoff, therefore a

special effort was made to have the initial STEC abundance levels as equivalent as possible. Enrichment methods were used to assess whether any viable STEC strains (or their genes) that remained in the simulated runoff showed clear STEC strain differences (Table 2). Month of last culture-based and PCR-based detections ranged from 4 to 30+ months. Comparing detection methods, STEC in microcosm samples remained detectable from 2 to 12 months longer using the PCR-based detection of serotype-specific genes compared with the culture-based approach. However, some STEC strains (O45, O121, and O145) demonstrated no difference between culture-based and PCR-based detection. The correlation between culture- and PCR-based methods was quite strong ( $r=0.891$ ).

The rates of  $\log_{10}$  abundance change per month during the incubation (month 1 to month 12) ranged from  $-0.150$  to  $-1.995 \log_{10}$  (Table 2). There were significant differences ( $p < 0.05$ ) between the strains, with the longest surviving strains (O26, O45, O103, and O157) having the lowest rates of decline and shortest surviving strains (O111, O121, and O145) having the highest rates of decline. Correlation between the rate of change in abundance and interval required for a 3- $\log_{10}$  reduction was moderate ( $r=0.535$ ). This aspect of survival is expected to be insensitive to initial STEC abundance and may be a better means for comparison among independent studies.

Due to the striking difference between the survival attributes of STEC O26 and O45 strains compared with the more sensitive O121 and O145 strains, the experiment was repeated for these isolates in a second year of the study. Similar results were observed (Fig. 3). First, both O26 and O45 demonstrated an initial increase in abundance during the first month of incubation. While STEC O26 and O45 were culturable through month 12, O121 and O145 were no longer detectable by month 2. Enrichment, followed by culture-based and PCR-based detection, was also consistent with the prior study. Continued examination of the year 1 microcosms resulted in continued culture of STEC O26, O45, and O103 and detection of O157 at 18 months. Finally, although the

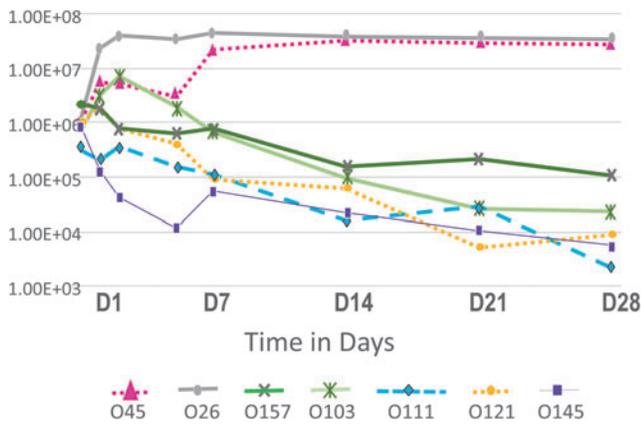
**TABLE 2.** SUMMARY OF LOG ABUNDANCE (PER mL) AND COMPARISONS OF SURVIVAL/PERSISTENCE OF SHIGA TOXIGENIC *ESCHERICHIA COLI* SEROTYPES IN SIMULATED FEEDLOT RUNOFF

Strain	STEC abundance				3-Log reduction, days	Month of last detection <sup>a</sup>		Rate of change, log/month <sup>b</sup>
	Initial	Peak	p	Peak day		Culture based	PCR based	
O26	6.076	7.663	<0.001	7	517 A	26 B	30 + A	-0.150 A
O45	6.027	7.502	<0.001	14	576 A	30 + A	30 + A	-0.158 A
O103	5.834	6.810	0.003	2	40 C	18 C	20 + B	-0.265 A
O111	5.482	5.524	0.792	2	37 C	5 D	19 C	-0.919 B
O121	6.024	6.232	0.186	1	39 C	4 D	4 C	-0.843 B
O145	5.951	5.951	1.000	0	40 C	5 D	5 C	-1.995 C
O157	6.339	6.339	1.000	0	168 B	18 C	20 B	-0.319 A
LS Mean SE					26	1	1	0.148

<sup>a</sup>For determining the month of last detection, 1 mL of the simulated runoff was enriched in 9 mL of TSB + nalidixic acid, followed by plating. Culture-based determination examined colonies for serotype-specific PCR confirmation on the plate after enrichment, while PCR-based determination examined the enrichment broth directly for serotype-specific genes. For statistical purposes, strains present at the end of the experiment were assigned either month 30 or 20 as the month of last detection, possibly underestimating their actual survival. Strains sharing a common postscript do not differ ( $p < 0.05$ ) in the month of last culture, 3-log reduction, or PCR-based detection.

<sup>b</sup>Rate of log abundance change per month calculated from month 1 to 12. Strains sharing a common postscript do not differ ( $p < 0.05$ ) in their rates of change in abundance.

PCR, polymerase chain reaction.

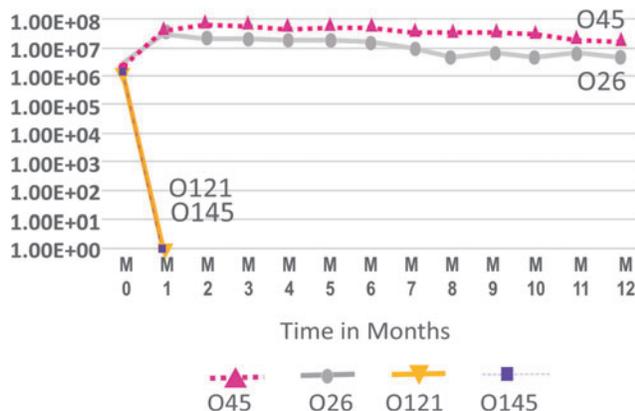


**FIG. 2.** Short-term enumeration of culturable cells over time (first 28 d). STEC cells were plated on Day 1, Day 2, Day 5, Day 7, Day 14, Day 21, and Day 28. Three strains (O45, O26, and O103) initially grew upon addition to microcosms. Nomenclature: (D) denotes day; STEC strains are listed by somatic (O) antigen. STEC, Shiga toxin-producing *Escherichia coli*.

rates of log<sub>10</sub> abundance change for STEC O26 and O45 were lower in the second trial (−0.092 and −0.046) compared with the first trial, the rates were not statistically different ( $p > 0.05$ ) from the first trial.

## Discussion

Environmental survival of pathogenic STEC is an important consideration when estimating the likelihood of adverse outcomes from contact with contaminated water or soil. It is also useful to inform decisions regarding remediation and control measures following contamination with manure-borne STEC. In these experiments, STEC growth by some strains was observed when added to feedlot runoff microcosms. This initial increase in CFU numbers is consistent with generic *E. coli* growth patterns observed in field studies of land-applied manure slurries (Meyers *et al.*, 2020) and is supported by evidence that some strains of *E. coli* survive for extended periods of time outside of the GIT or even natu-



**FIG. 3.** Replication of the study for selected STEC strains: to confirm initial results, the full microcosm experiment was run a second time (year 2) for selected strains. Nomenclature: (M) denotes month; STEC strains are listed by somatic (O) antigen. STEC, Shiga toxin-producing *Escherichia coli*.

ralize in the extraintestinal environment (Jang *et al.*, 2017). Evolution experiments performed by Clarke *et al.* (2020) with generic *E. coli* demonstrate clear genotype  $\times$  environment effects for *E. coli* and note strain-level differences in the response of *E. coli* to a variety of environmental variables. In the current study of STEC survival potential, focusing on the top 6 non-O157 STEC strains under strictly restricted nutrient concentrations, we noted a consistent response between the three replicates for each strain/serotype, but heterogeneity in the responses between strains.

A growing body of evidence from this and other studies (Free *et al.*, 2012; Ma *et al.*, 2014; Jang *et al.*, 2017) demonstrates a broad range of phenotypes among STEC strains involving extraintestinal survival, with some serotypes appearing to be inherently more robust. Microcosm results from this investigation using strains from seven O157 and non-O157 serotypes showed that some STEC strains have the biological potential to survive and remain culturable in manure-impacted waters for as long as 18 months. There also appear to be nonrandom serotype-specific differences in survival of target bacteria and persistence of target genes in the set of samples examined in this investigation.

The experimental design of this study does not allow for broad serotype-specific conclusions to be drawn; however, data presented here are consistent with other reports of serotype-specific differences in STEC prevalence and survival outside of the gut. For example, as it relates to environmental survival, Stromberg *et al.* (2018) reported that the O26 serotype is the most prevalent non-O157 STEC on beef cattle hides, and Dixon (2020) found O45 to be the most prevalent non-O157 STEC in cattle feces. Ma *et al.* (2014) found STEC O103 persisting longer than STEC O157 in soil, and Bezanson *et al.* (2019) determined strain-specific differences in persistence on plants, with STEC O103 persisting longer and surviving better than other strains. In this study, the STEC O26 and O45 strains remained culturable above the  $10^5$  CFUs/mL range for the duration of the original 12-month study and remained culturable through months 25 and 30, respectively. The STEC O157 strain remained culturable through month 17. However, STEC O111, O121, and O145 declined rapidly following inoculation into simulated cattle feedlot runoff. Unexpectedly, the STEC O111 microcosms remained positive by PCR *rfbO111* months after bacteria could no longer be recovered. It is possible that the DNA was protected in living, but not culturable, cells or that interactions with soil stabilized DNA from dead or lysed cells (Nagler *et al.*, 2018). Together, these data highlight that the biological potential of non-O157 in the environment might be most accurately modeled using serotype-specific data. However, in all instances, studies using many more strains of each serotype are needed before any serotype-specific approaches can be recommended.

Under field conditions, the survival potential of STEC is tempered by many factors not accounted for in the current set of microcosm experiments. The composite of macroecological elements such as temperature, precipitation, and sunlight; microecological elements such as pH; and other localized physical, chemical, and biological parameters results in a complex collection of selection pressures that impact the survival of individual bacterial taxa. Competition with the native manure, soil, and waterborne microbial communities and predation by micro- and mesofauna can

influence the persistence of STEC in the environment in a site-dependent manner (Ma *et al.*, 2014; Moynihan *et al.*, 2015). The long survival times observed in this study would not be expected under field conditions where the target organisms are subjected to fluctuating temperatures and must compete with the native microbial flora. Knowing the survival potential of target pathogens in the absence of a myriad of other selective forces provides a baseline for understanding more complex interactions in the natural environment.

## Conclusions

Food animal manure is an important source of STEC, and runoff from grazing areas, confined food animal operations, or land application sites can introduce STEC into surface waters. In the current study, STEC O26 and O45 strains appeared to be the most environmentally robust, remaining culturable for more than a year when found in simulated runoff. Results presented here support conclusions from other studies conducted in soil and food matrices, which demonstrated strain and serotype-specific differences in survival of STEC serotypes in the *E. coli* extraintestinal habitat. Serotype-specific differences in STEC environmental persistence should be considered when evaluating potential exposure from manure-impacted runoff.

## Author Contributions

L.M.D. was involved in conceptualization, project management, methodology, analysis, writing—original draft preparation, and writing—reviewing and editing. J.E.G. was involved in methodology, analysis, and writing—reviewing and editing. D.N.M. was involved in methodology, data curation, statistical analysis, and writing—reviewing and editing.

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## Disclaimer

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## Disclosure Statement

No competing financial interests exist.

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## Supplementary Material

Supplementary Table S1  
Supplementary Table S2

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