

Developing a Simple Strategy for Roadside Spring Water Disinfection in Central Appalachia

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Abstract: Several studies have highlighted issues of unreliable access to safe drinking water in the Appalachian region. In some cases, residents turn to roadside springs as a practical, and culturally valuable, drinking water source. However, public reliance on roadside springs for potable use can present concerns, as bacterial contamination of spring water has been documented throughout Appalachia. This study aimed to 1) develop a simple, low-cost protocol using household bleach to inactivate total coliform and *E. coli* in untreated roadside spring water; 2) provide educational materials at local roadside springs to inform users of this simple treatment strategy; and 3) assess spring user perceptions of the educational materials via a short survey. Laboratory scale trials emulating typical spring water collection and storage conditions investigated the use of household bleach (7.4-7.5% sodium hypochlorite) for total coliform and *E. coli* bacteria inactivation and free chlorine residual maintenance in spring water over time. Results showed that 2 drops (approximately 0.10 mL) of household bleach from an eyedropper per 1 gallon of spring water provided adequate total coliform and *E. coli* disinfection, while maintaining free chlorine levels below typical taste thresholds and providing sufficient residual over a 1-month trial period. An infographic communicating the disinfection protocol and a corresponding survey were created and distributed at roadside springs in rural regions of southwestern Virginia and southern West Virginia. The majority of spring user survey respondents (80%) reported that the infographic was generally helpful, and over half of respondents stated that they would use the bleach protocol.

Keywords: *drinking water, spring water, disinfection, free chlorine, total coliform, E. coli*

One in four people worldwide do not have access to safely managed drinking water (Ritchie and Roser 2021). In the U.S., although nearly 100% of the population is reported to have access to safe drinking water, issues related to drinking water quality, reliability, cost, and equity of access persist (Allaire et al. 2018; Mueller and Gasteyer 2021). Though the specifics of any environmental health concern often include locally unique aspects, problems with safe drinking water access in the U.S. are generally clustered in regions or communities characterized by similar contextual factors observed in struggling regions across all nations. For example, in Appalachia, a mountainous 531,000 square-kilometer region

in the eastern U.S. (Pollard and Jacobsen 2021), the maintenance of essential water infrastructure is limited by socioeconomic, geographic, and geotechnical challenges (Cook et al. 2015; Arcipowski et al. 2017). Though shaped by different nation-specific histories, multiple authors have noted that factors such as steep topography, high poverty rates, and unique geography in rural mountainous regions present difficulties in the provision of safe drinking water and appropriate wastewater treatment (Browne et al. 2004; Wescoat et al. 2007).

Not surprisingly, rural mountainous regions often lag behind non-mountainous regions in the establishment and maintenance of drinking water

Research Implications

- Roadside spring water, while consistently testing positive for total coliform and *E. coli*, is often utilized as a drinking water source in the Appalachian region.
- Under conditions mirroring those used to collect and store roadside spring water in the Appalachian region, 2 drops of regular, unscented household bleach (7.4-7.5% sodium hypochlorite) successfully deactivated total coliform and *E. coli* in 1 gallon of roadside spring water and maintained a free chlorine residual of between 0.2 mg/L and 2 mg/L for the 1-month trial period.
- Efforts to provide information on spring water quality and bleach disinfection via infographic were considered to be helpful, however, only half of survey respondents reported that they intended to use the bleach disinfection protocol.
- Feedback suggests that while the infographic may be a useful tool in addressing roadside spring water use, broader discussion of risks associated with spring water use and efforts to improve in-home piped water infrastructure are necessary to reduce health risks.

infrastructure, and the Central Appalachian region is no exception (Browne et al. 2004; Wescoat et al. 2007). In some regions of Central Appalachia, upwards of 10% of homes lack complete plumbing (Krometis et al. 2017). While more recent data are not yet available, in 2005, 75% of the population in the Appalachian region had access to community water systems, behind the national level of 85% (Hughes et al. 2005). *Community water systems*, private or publicly owned systems providing piped drinking water to at least 15 service connections or that serve at least 25 people, are regulated by the U.S. Environmental Protection Agency's (EPA) Safe Drinking Water Act (SDWA; Tiemann 2017). Homes in Appalachia without access to community water systems most commonly rely on private wells for in-home water. *Private wells*, groundwater systems that serve no more than 25 people at least 60 days of the year and have fewer

than 15 service connections, do not fall under the purview of the SDWA (CDC 2014). Private well systems generally do not employ treatment (Smith et al. 2014) and, as a result, the presence of health-based and aesthetic contaminants in private well water at the point-of-use (POU) is common (Shiber 2005; Pieper et al. 2015; Law et al. 2017; Patton et al. 2020). Issues with POU water quality are not confined to homes with private wells. Indeed, small, rural community water systems often struggle to comply with the SDWA regulations due to limited financial, technical, and human resources available for operation and maintenance needs (Hughes et al. 2005; Allaire et al. 2018; Marcillo and Krometis 2019).

Multiple studies report that Appalachian households without adequate access to drinking water of sufficient quality and quantity rely on alternative sources to satisfy daily potable water needs (Blakeney and Marshall 2009; McSpirit and Reid 2011; Arcipowski et al. 2017; Page et al. 2017; Krometis et al. 2019; Patton et al. 2020; Cohen et al. 2022). Though often preferred by homeowners (Blakeney and Marshall 2009; Arcipowski et al. 2017), reliance on bottled water presents a costly alternative in a region where the mean per capita income is significantly lower than the national average (Pollard and Jacobsen 2021). Given a lack of confidence in household water and the cost of alternatives, some residents of the Appalachian region collect a portion, or all, of their household drinking water from roadside "spout" springs, i.e., unimproved and untreated environmental waters often located along roads where road cuts have intersected with shallow groundwater aquifers or where mine pools are discharging (Swistock et al. 2015; Krometis et al. 2019; Patton et al. 2020; Sinton et al. 2021). Notably, recent surveys of regular roadside spring users revealed that the majority have access to in-home tap water but perceive roadside spring water to be of higher quality (Krometis et al. 2019; Patton et al. 2020). Although it is difficult to quantify the total population dependent on these water sources, Swistock et al. (2015) reported that 30% of Pennsylvania residents attending local Cooperative Extension programming had consumed water from a roadside spring at some point, and that 12% consumed spring water annually.

Reliance on roadside springs for potable use does present some concern, as bacterial contamination appears ubiquitous across regions. Krometis et al. (2019) reported that 99% (n = 83) of roadside spring samples collected from 21 springs in five Central Appalachian states were positive for total coliform bacteria and 86% of samples were positive for *E. coli*. Swistock et al. (2015) detected not only total coliform and *E. coli* in seasonal samples collected from Pennsylvania roadside springs (n = 37), but also *Giardia* and *Cryptosporidium* contamination in a small subset of samples (n = 10). This is especially concerning given previous reports of *Giardia* outbreaks linked to consuming roadside spring water in New York (Bedard et al. 2016). A broader examination of roadside springs throughout western New York determined that 86% of springs failed at least one U.S. EPA SDWA drinking water standard, most often for fecal indicator bacteria, which presents an immediate health risk (Sinton et al. 2021).

Despite the likelihood of poor or inconsistent water quality, it is critical to recognize that these springs, and the collection of spring water, is a common practice and is culturally valued in many rural Appalachian communities (Westhues 2017; Krometis et al. 2019; Patton et al. 2020). More importantly, in a study directly comparing the home water quality of spring users in some Central Appalachian communities with water from their preferred roadside spring, Patton et al. (2020) reported that in many cases, increased concentrations of metals associated with taste and aesthetic issues (e.g., iron, manganese) were much more common in tap water than spring water. Given that household POU options may be less palatable, the expense of bottled water, documented preference for the aesthetic qualities of spring water, and the associated cultural significance of roadside springs, it is not surprising that efforts to close access points to roadside springs can be met with resistance (Bedard et al. 2016; Williamson 2018).

The U.S. EPA and the Centers for Disease Control and Prevention (CDC) recommend the use of bottled water, boiled water, or bleach-disinfected water in situations where regular water service is interrupted and/or water may be unsafe to drink due to the presence of fecal indicator bacteria (U.S.

EPA 2017; CDC 2021). As previously mentioned, bottled water is a costly drinking water alternative that some in the Appalachian region are unable to afford. Moreover, as noted in multiple studies (Swistock et al. 2015; Krometis et al. 2019; Patton et al. 2020; Sinton et al. 2021), many spring users collect water in bulk and may be unable or unwilling to boil all the spring water before using it. In developing countries, chlorination of drinking water via sodium hypochlorite has been found to be a successful method of disinfecting drinking water that helps to decrease levels of coliform in drinking water (Sobel et al. 1998; Firth et al. 2010) and related diarrheal episodes in homes (Quick et al. 1999). Sodium hypochlorite, the active ingredient in chlorine bleach, is a well-established disinfectant that has been used since the 1820s as a disinfecting and bleaching agent (Ponzano 2007). The CDC and the Pan American Health Organization (PAHO) have developed a chlorine-based intervention called Safe Water System that utilizes sodium hypochlorite (the active ingredient in bleach) for in-home disinfection of drinking water (CDC 2003). The present study aimed to: 1) develop a simple, low-cost protocol using household bleach to inactivate total coliform and *E. coli* in untreated roadside spring water; 2) provide educational materials at local roadside springs to inform users of this simple treatment strategy; and 3) assess spring user perceptions of the educational materials via a short survey. Laboratory scale trials emulating typical water collection and storage investigated the use of name-brand and store-brand household bleach in varying quantities to determine effectiveness in *E. coli* bacteria inactivation and maintenance of free chlorine residual in spring water over time.

Methods

Disinfection Protocol Design

Four 1-month trials were completed between August and October of 2021 assessing the effect of household bleach brand and volume on levels of total coliform, *E. coli*, free chlorine, and total chlorine in locally collected spring water samples (Table 1). The 1-month trial duration was chosen because previous studies documenting roadside spring use found that most surveyed spring users

Table 1. Variables assessed in each of the four trials of bleach disinfection of roadside spring water completed. NB = name-brand, SB = store-brand.

Trial Number	Bleach Brand	Bleach Treatment Volumes (per 1 gallon of water)	Number of Samples	Spring Sampled	Trial Duration
1	NB	0 tsp, 1/4 tsp	8	Spring 1	1 Month
2	NB	0 tsp, 1/8 tsp, 1/16 tsp	12	Spring 1, Spring 2	1 Month
3	NB	0 drops, 3 drops, 2 drops, 1 drop	4	Spring 1	1 Month
4	NB, SB	0 drops, 2 drops	6	Spring 1	1 Month

collected water from springs at least once a month (Krometis et al. 2019; Patton et al. 2020). Based on this information, one month was considered a realistic amount of time that an individual may store spring water at their home before running out and/or collecting a fresh batch. Trials included assessment of one type of unscented name-brand bleach (Clorox) and one type of unscented store-brand bleach (Dollar General). The brands were chosen based on the types of chlorine-containing household bleach that are readily available at commercial stores in southwest Virginia and southern West Virginia.

Bleach volumes tested varied from 1/4 tsp (~ 0.6 mL) to 1 drop per 1 gallon of roadside spring water. Drops were dispensed from a disposable plastic eyedropper designed to distribute approximately 0.05 mL per drop (Table 1). While the CDC recommends the addition of 1/4 to 1/8 tsp (~ 0.6 to 1.2 mL) of unscented household bleach per 1 gallon of water, the U.S. EPA recommends 6 to 8 drops (~ 0.3 to 0.4 mL) of unscented household bleach per 1 gallon of water (U.S. EPA 2017; CDC 2021). The objective of assessing different bleach volumes was to determine the smallest quantity of bleach that could be added to spring water that would both inactivate total coliform and *E. coli* bacteria and provide a suitable free chlorine residual to protect water that is being stored for longer than 24 hours, as previous studies suggest that spring water is often stored for multiple weeks (Krometis et al. 2019; Patton et al. 2020). The CDC recommends free chlorine residual levels between 0.5 and 2.0

mg/L one hour after disinfection, and greater than 0.2 mg/L 24 hours after disinfection (CDC 2020). Individuals can taste or smell chlorine in drinking water at concentrations well below 5 mg/L, and even as low as 0.3 mg/L (Cridler et al. 2018; WHO 2022). The study aimed to maintain a residual below 2.0 mg/L as this is considered the taste threshold for free chlorine (CDC 2020) and spring users frequently cite the taste of spring water as a reason for preferring it over their home drinking water (Swistock et al. 2015; Krometis et al. 2019; Patton et al. 2020; Sinton et al. 2021). A primary concern is that if an excessive quantity of bleach is added to the spring water and impacts taste, spring users may decide not to follow the dosing regimen to disinfect their spring water—and potentially opt for no treatment at all.

Spring Selection and Sample Collection

For each trial, water was collected from a roadside spring in Virginia (Spring 1) that is regularly used by local residents for potable water (Table 1). This spring consistently tested positive for total coliform and *E. coli* in a previous study, with maximum detected levels of 2,149 MPN/100 mL and 583 MPN/100 mL, respectively (Krometis et al. 2019). Compared to the 21 roadside springs previously sampled, water samples collected from this spring yielded the highest recorded total coliform and *E. coli* levels in the study (Krometis et al. 2019). For trial 2, water was also collected from a second roadside spring in West Virginia (Spring 2) which, while less frequently positive

for fecal indicator bacteria (Patton et al. 2020), is another known popular location for the collection of drinking water. The turbidity of spring water collected and tested during this study was between 0.0 and 0.13 NTU, which is considered low (USGS 2018).

Sample collection and storage were designed to emulate the practice of spring users collecting roadside spring water as closely as possible. All bleach dosing trials were conducted in spring water collected and stored in one-gallon plastic milk jugs (Figure 1). Anecdotally, these vessels are commonly used for the collection and storage of spring water for drinking (Figure 1). Prior to spring water collection, plastic jugs were washed with dish soap and tap water to simulate what would be available for cleaning in a home. Each jug was rinsed three times with spring water prior to collecting samples to eliminate any residual chlorine that may have been present in the tap water used for cleaning. After collecting the spring samples, the plastic jugs were capped and transported to the laboratory for immediate analysis. Throughout the study, spring

water-filled jugs were capped and stored on a countertop out of direct sunlight in a $\sim 20^{\circ}\text{C}$ room, in keeping with common spring water storage conditions in households (Figure 1). Additionally, samples were not transported on ice because spring water is not commonly iced when transported by local residents.

Water Analyses

Upon returning to the lab and prior to the addition of bleach, spring samples were tested for bacteriological contaminants and initial chlorine levels. After initial testing, bleach was added to the sample using either a 1/4 or 1/8 tsp kitchen measuring spoon, or a plastic eyedropper (Thermo Fisher Scientific, Waltham, MA). After the addition of bleach, the water jugs were inverted three times. Following inversion, bacteria and chlorine levels were measured at 5-minute, 30-minute, 1-day, 1-week, and 1-month post-bleach intervals. In each trial, control samples were not dosed with bleach. The 5-minute, 30-minute, and 1-day post-bleach measurement intervals were selected to determine



Figure 1. Left – Collected roadside spring water stored in one-gallon plastic jugs in the home of a spring user in Central Appalachia. Right – Roadside spring water sample being collected for this study in a one-gallon plastic milk jug (original photos).

the minimum reasonable contact time required for disinfection. One-week and 1-month post-bleach intervals were selected because multiple authors report spring users collecting water at least once a month and often, once a week (Krometis et al. 2019; Patton et al. 2020; Sinton et al. 2021). These time periods also allowed for the investigation of potential bacterial regrowth during prolonged storage.

Samples were tested for free and total chlorine before bleach treatment and at the post-bleach treatment time intervals using a HACH DR300 Pocket Colorimeter (HACH, Loveland, CO). Bacteriological analysis of spring water samples before and after bleach treatment was completed for total coliform and *E. coli* via the Colilert-defined substrate method (www.idexx.com, Westbrook, MN).

Infographic Creation and Distribution

After the disinfection protocol design process was completed, an infographic (Figure 2) featuring spring water quality information and the disinfection protocol was created for distribution at five local roadside springs in southwestern Virginia and southern West Virginia that are frequently used for drinking water collection. The front side of the infographic features information on the potential for spring water to contain bacteriological contaminants, as well as a link to a public website with recent bacteriologic data from the spring. The front side of the infographic also included a link to a website that provides periodically updated water quality reports for local springs that have been

sampled. The back side of the card features the simple bleach protocol to disinfect spring water. The goal during infographic development was to provide spring users with useful information about spring water quality and disinfection delivered in an objective, easily accessible, and discreet manner. Local contacts reviewed the language to ensure it was not inflammatory and was easy to understand.

Postcard-sized infographics were printed and laminated so that the spring users could keep the infographic for an extended period of time. Infographics were placed in a plastic sandwich bag along with one disposable plastic eyedropper (i.e., the same used in all trials) so that spring users would have access to an appropriate tool to help measure out the correct bleach quantity. Additionally, a postcard-sized, pre-addressed anonymous survey was included in the bag. The bags were distributed in plastic bins at five local springs beginning in December of 2021 with the goal of continuing distribution every few months.

Survey Development and Distribution

To assess spring user response to the infographic, a brief anonymous survey was designed (Figure 3). The survey included questions regarding spring use, previous knowledge of spring water quality, and reception of the infographic. Questions were kept brief, anonymous, and easily understood so that spring users would be more inclined to complete and return the survey. The purpose of the survey was not to further examine motivations

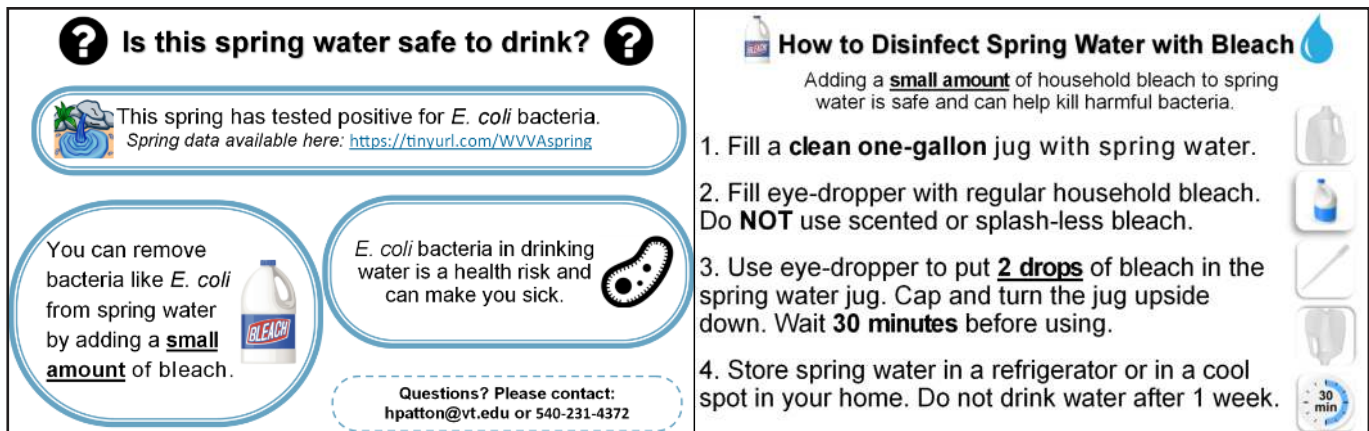



Figure 2. Left – The front side of the infographic featuring information on spring water quality. Right – The back side of the infographic featuring the protocol for bleach disinfection of spring water.

We Want to Hear from You!

Please answer the following questions:

Answer online at:
<https://tinyurl.com/RoadsideSpringSurvey>



1. What do you use spring water for? Please check all that apply.
 Drinking Cooking Cleaning Brushing teeth Farming/Gardening
 Livestock/Pets Other: _____

2. Did you know that spring water can have harmful bacteria in it?
 Yes No Other: _____

3. Do you already disinfect your spring water? If yes, how?
 Yes, boiling Yes, chlorine Yes, other: _____ No

4. Will you use the instructions for bleach disinfecting your spring water?
 Yes No Maybe Other: _____

5. How helpful did you find this information?
 Very helpful A little helpful Not helpful Other _____

Please write any other comments or suggestions that you have on the back.

Figure 3. Postcard-sized, pre-addressed anonymous survey distributed with infographic and plastic eyedroppers at local roadside springs.

for spring use, but to assess the success of the infographic in providing useful information to spring users. As previously mentioned, the survey was postcard-sized, included in plastic sandwich bags with the infographic and a plastic eyedropper, and distributed at five roadside springs local to southwestern Virginia and southern West Virginia. The survey included a return address and postage as well as a link to an online version of the survey. This allowed spring users to choose the survey format (paper or online) that was more convenient for them to complete and return.

Results and Discussion

Chlorine Residual

As previously stated, a specific study aim was to determine the minimum volume of household bleach that could be added to 1 gallon of spring water to inactivate total coliform and *E. coli* bacteria and maintain a free chlorine residual of between 0.5 and 2.0 mg/L 1-hour post-disinfection, and at least 0.2 mg/L 1-day post-disinfection, in accordance with CDC (2020) recommendations. Identifying the minimum quantity necessary for addition would ensure that taste and/or aesthetic issues were minimized, thus maximizing the potential adoption of this strategy by regular spring users.

Spring water dosed with CDC recommended bleach quantities of 1/4 and 1/8 tsp per 1 gallon (~ 3.79 L) of water yielded free chlorine levels of at least 8.8 mg/L throughout the duration of the trial time period, the maximum measurement range for the HACH colorimeter (Figure 4). Exceedance of the free chlorine residual taste threshold (2.0 mg/L) is of particular concern, given repeated reports that spring users prefer the taste of spring water to home or alternative drinking water sources (Swistock et al. 2015; Krometis et al. 2019; Patton et al. 2020). Halving the 1/8 tsp recommendation to 1/16 tsp bleach still yielded free chlorine levels that reached the maximum measurement range of 8.8 mg/L for the duration of the trial period.

The bleach volume of 1 drop from a plastic eyedropper (0.05 mL) successfully maintained a free chlorine residual below 2.0 mg/L for the duration of the trial period. However, 1-week post-disinfection the residual fell to 0.17 mg/L, below the CDC recommended range. This is of concern because previous studies regarding roadside spring water use found that individuals often collect spring water weekly or monthly, suggesting that collected spring water may be stored for several weeks prior to being used (Swistock et al. 2015; Krometis et al. 2019; Patton et al. 2020; Sinton et al. 2021). Bleach volumes of 2 and 3 drops from a plastic eyedropper (0.10 and 0.15 mL, respectively)

yielded free chlorine levels that were greater than zero but less than the maximum detection limit, for the duration of the trial time period. While the free chlorine residuals for 2 and 3 drops exceeded the CDC recommended maximum of no greater than 2 mg/L at the 5-minute and 30-minute post-disinfection time points, at the 1-day post-disinfection time point, the free chlorine residual of the 2 drop trials decreased to 2.0 mg/L. This residual remained within the CDC recommended range of 0.2 to 2.0 mg/L for the remainder of the trial duration.

Lantagne et al. (2014) analyzed the performance of the U.S. EPA recommended dose of bleach for drinking water disinfection in the event that bottled or filtered water is unavailable (approximately 1/8 tsp or 8 drops in 1 gallon (~ 3.79 L) of water). Similar to the present study, the authors dosed water collected in one-gallon vessels, but utilized water from various sources including tap water, surface water, well water, and rain barrels. The authors determined that 24-hours after dosing, 81% of samples dosed with 2 mg/L of sodium hypochlorite fell between the desired free chlorine residual range of 0.2 mg/L (the CDC recommended minimum free chlorine residual level) and 4 mg/L (the SDWA Maximum Contaminant Level for free chlorine). Sodium hypochlorite doses of 4 and 7

mg/L resulted in only 69% and 14% of samples remaining within the desired free chlorine residual range, respectively, suggesting that these doses are too high. Lantagne et al. (2014) concluded that existing U.S. EPA recommendations for the chlorine disinfection of drinking water need to be adjusted to lower, more accurate doses, especially given recent chlorine increases in commercially available bleach. The Lantagne et al. (2014) results are consistent with the results of the present study where 2 drops (0.10 mL) of bleach in 1 gallon of spring water, equivalent to approximately 1.95 mg/L of sodium hypochlorite, provided a sufficient chlorine residual of between 0.2 and 2 mg/L 24-hours after dosing.

Notably, after determining that 2 drops of bleach provided an appropriate free chlorine residual within the desired range for 1 gallon of spring water, an additional trial was completed testing the volume using name-brand and store-brand bleach to account for any differences in chlorine strength. Bleach brand testing was completed due to concerns about varying chlorine levels in commercially available bleach (Lantagne 2009). In the current study, differences were negligible (see Supplementary materials, Figure S1). Figure 4 represents results from the name-brand bleach trials. Both name- and store-brand bleach

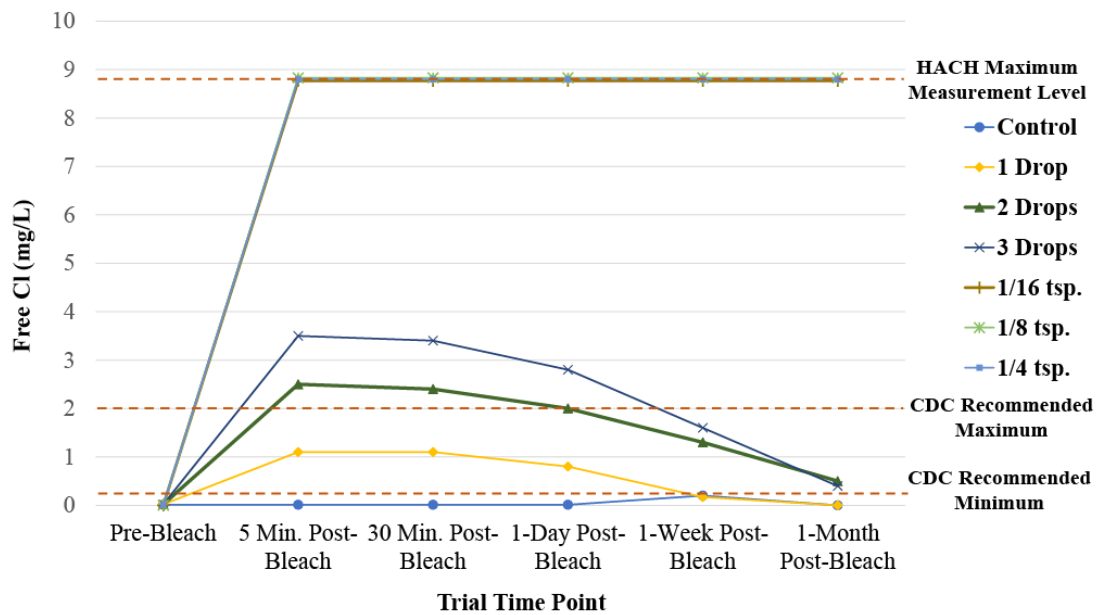


Figure 4. The relationship between bleach dosage (per 1 gallon of spring water) and free chlorine measured in collected spring water over time. (n = 2 for 1/4 tsp, 1/8 tsp, 1/16 tsp; n = 5 for 2 drops; n = 1 for 1 drop and 3 drops).

effectively inactivated total coliform and *E. coli* in spring samples for the duration of the trial period, and both provided a sufficient chlorine residual.

Total Coliform and *E. coli*

All quantities of added bleach at or above 2 drops per gallon successfully inactivated total coliform and *E. coli* in the spring water and prevented regrowth for the duration of the study (Figures 5 and 6). As all coliform levels in samples prior to the addition of bleach exceeded 1000 MPN/100 mL, this represents an approximately 2-3 log-scale inactivation. Total coliform appeared to regrow when an insufficient residual (below 0.2 mg/L) was maintained, given detection at 1-week and 1-month post-disinfection in the spring water treated with 1 drop of bleach. *E. coli* levels in pre-disinfection spring samples were notably lower than that of total coliform (1.5 to 54.5 MPN/100 mL), which aligns with typical observations at roadside springs in this region (Krometis et al. 2019), and no *E. coli* regrowth was observed under any of the bleach regimes. As 2 drops of bleach appeared effective both in inactivation of fecal indicator bacteria and maintenance of an appropriate residual below the CDC (2020) taste threshold, this quantity was selected for infographic recommendation.

Survey Results

Ten individuals responded to the surveys left at the five springs. The majority (70%) reported using the roadside spring water for drinking and cooking. Survey respondents also reported using roadside spring water for cleaning (30%), brushing teeth (30%), and for use with livestock/pets (50%). When asked whether they had previous knowledge of the potential presence of harmful bacteria in spring water (Figure 7), survey responses were split relatively evenly between yes and no. Despite 50% of survey respondents stating that they knew that roadside spring water could have harmful bacteria in it, 80% of respondents reported that they do not disinfect their spring water prior to using it.

With regards to infographic reception, 70% of respondents found the infographic content to be very helpful, 20% found the content to be not helpful, and 10% found the content to be a little helpful (Figure 8). Additionally, 60% of respondents stated that they would use the provided instructions for bleach disinfecting their spring water, 10% reported they would not use the instructions, and 10% reported they might use the instructions (Figure 8). One individual reported that they may reconsider use of roadside spring water entirely while another individual was skeptical about the safety of adding bleach to drinking water.

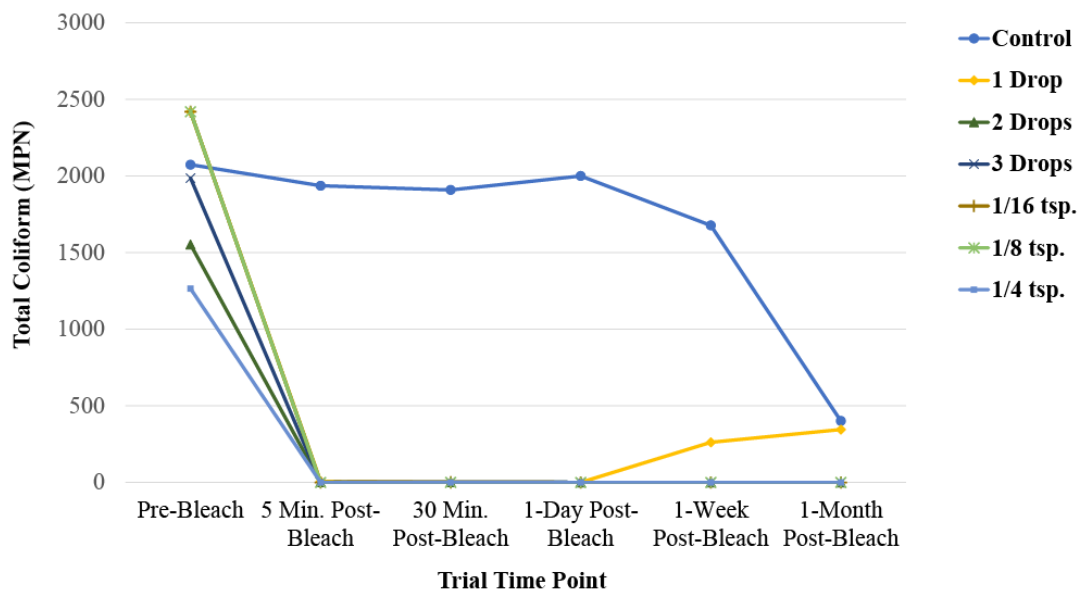


Figure 5. The relationship between bleach dosage (per 1 gallon of spring water) and total coliform bacteria measured in collected spring water over time. (n = 2 for ¼ tsp, 1/8 tsp, 1/16 tsp; n = 5 for 2 drops; n = 1 for 1 drop and 3 drops).

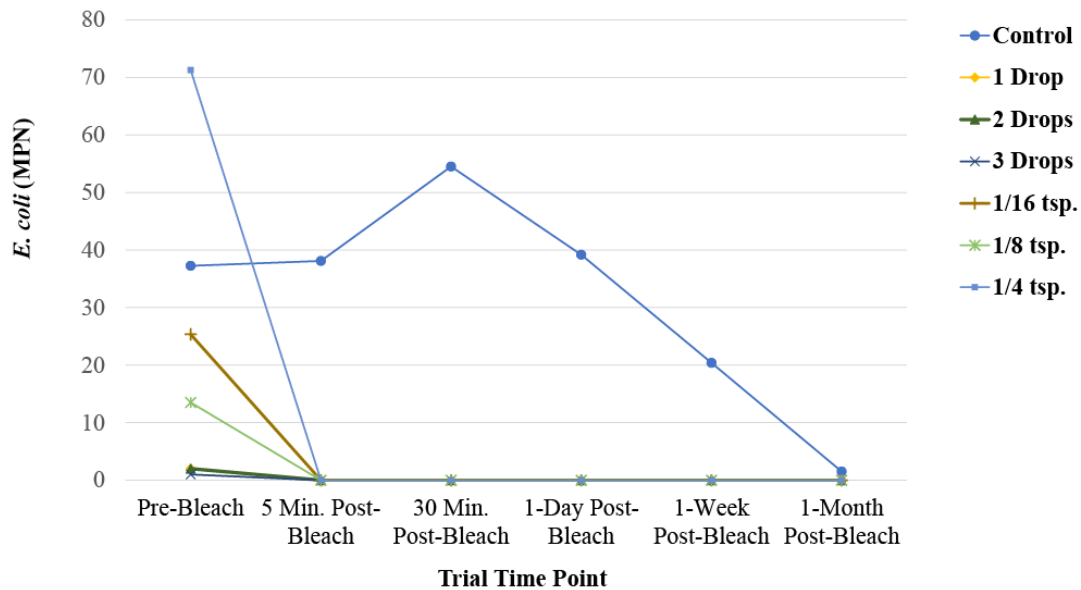


Figure 6. The relationship between bleach dosage (per 1 gallon of spring water) and *E. coli* measured in collected spring water over time. (n = 2 for ¼ tsp, 1/8 tsp, 1/16 tsp; n = 5 for 2 drops; n = 1 for 1 drop and 3 drops).

Though most survey respondents reported finding the infographic at least a little helpful, only a little over half of survey respondents stated that they would use the provided disinfection instructions and eyedropper. This feedback, and the individual response highlighting that spring users may be unsure of the safety of adding bleach to drinking water, suggests that the infographic may need more information on the safety of the addition of small amounts of bleach to drinking water, coupled with information on the dangers of adding too much bleach. Ideally, more widespread outreach and educational efforts in the area, discussing common water contaminants and health impacts, would encompass this information and information on home drinking water quality.

Limitations

We did not test bleach varieties with less than 7.4% sodium hypochlorite. Our disinfection protocol was developed using name-brand and store-brand household bleach with 7.4 to 7.5% sodium hypochlorite. This sodium hypochlorite concentration reflects the concentration of most commercially available regular household bleach products in the U.S. of between 5 and 9% (WS DOH 2015; CDC 2022). Certain varieties of commercially available household bleach, such as scented or splash-less products, have lower

sodium hypochlorite concentrations (1 to 5%). The infographic emphasizes the use of regular household bleach and not splash-less or scented varieties because of the higher concentration of sodium hypochlorite in regular household bleach.

Commercial household bleach has a shelf life of approximately six months, after which the product begins to degrade, becoming 20% less effective each year after it is produced (Ono 2006). The timeline of this study did not allow for the degradation of bleach to be factored into the scope of the experiment. The authors recommend that individuals interested in using bleach to disinfect their roadside spring water use new bleach bottles or bottles opened within six months and that have not exceeded any manufacturer printed expiration dates.

This bleach disinfection protocol is targeted at inactivating total coliform, *E. coli*, and other chlorine-vulnerable pathogens commonly found in spring water. Chlorine disinfection of drinking water is not effective in removing chlorine-resistant waterborne pathogens, such as *Cryptosporidium* (U.S. EPA 1999; CDC 2022), has low to moderate success in inactivating *Giardia* (CDC 2022), and thus should not be used in an attempt to disinfect drinking water that is suspected to be contaminated with chlorine-resistant pathogens. The inactivation of waterborne pathogens that are not bacteria, such as viruses, was also not tested during this

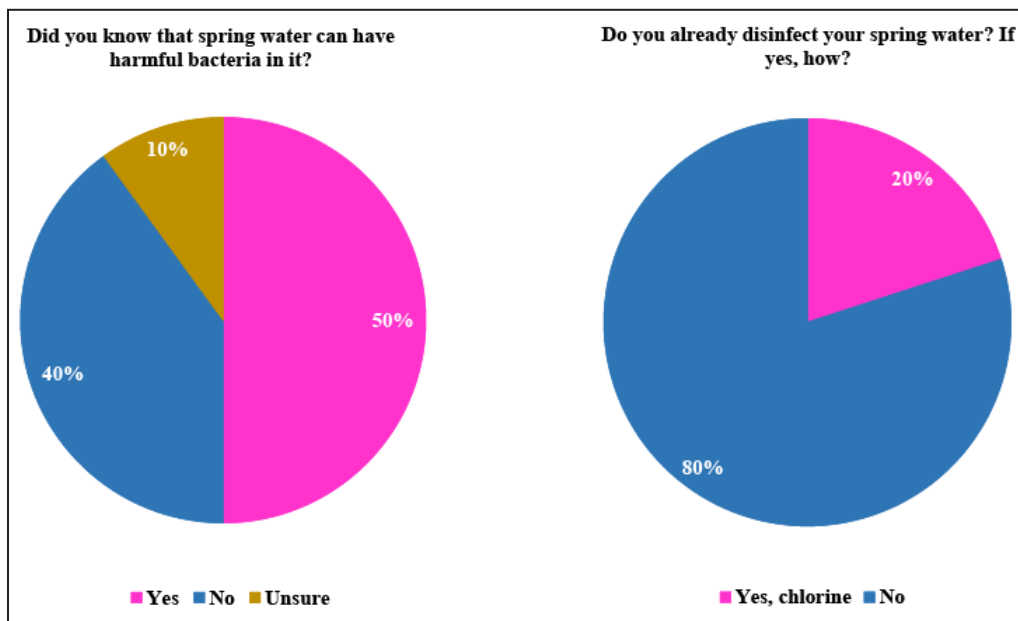


Figure 7. Survey responses collected regarding perception and use of roadside spring water.

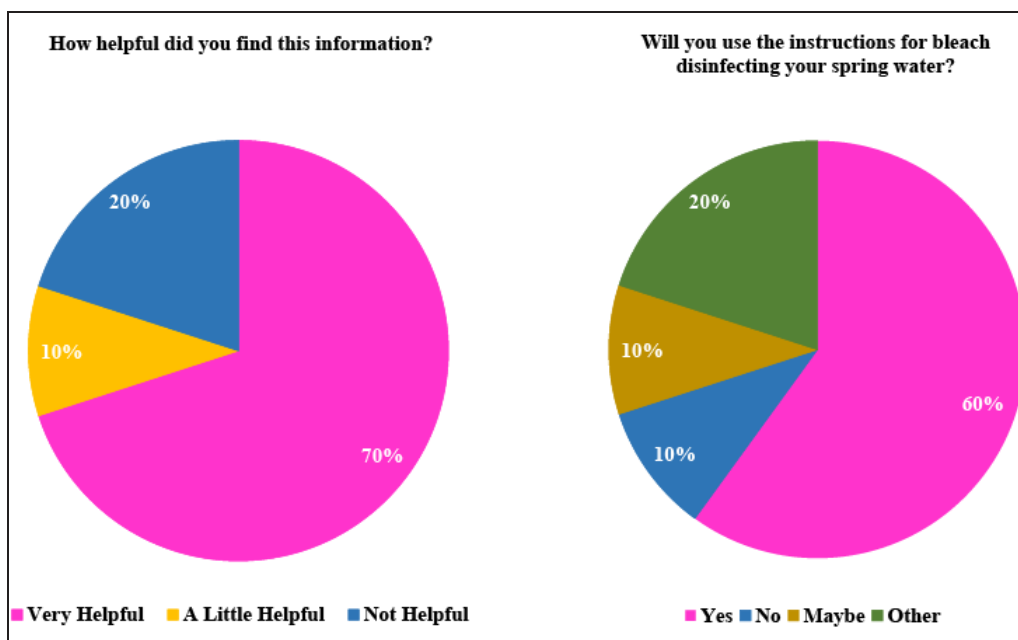


Figure 8. Survey responses collected regarding perception and use of the distributed infographic.

study. Additionally, it should be noted that bleach disinfection is less effective in turbid water (> 10 NTU; Crump et al. 2004; Lantagne 2008). The turbidity of the water tested was between 0.0 and 0.13 NTU. Turbidity values of less than 10 NTU in water are generally considered to be low (USGS 2018). For this reason, we feel that bleach disinfection of spring water in the Central Appalachian region can be an effective tool for the

inactivation of bacteria such as total coliform and *E. coli*. This disinfection method was not tested on spring water with higher turbidity, therefore, any future attempts to apply this method to spring water in other regions and/or to alternate water sources must consider water turbidity, among other water quality parameters.

Our survey sample from infographic distribution was convenience-based, as only spring users who

received and/or viewed the infographic information received the option to take the survey. However, based on the generally mixed responses to the infographic depicted in the survey data, we feel that it may be reasonable to draw general conclusions about infographic reception from the survey results. We do, however, caution against using this survey data to generalize spring users' reception to bleach disinfection, whether in Appalachia, across the entire United States, or in other countries.

Conclusion

Roadside springs are a common drinking water source for some households in the Central Appalachian region. Previous studies across multiple states indicate that users may prefer the taste and aesthetics of the spring water to other available drinking water sources, lack trust in their household drinking water, and/or lack access to in-home drinking water entirely. Based on previous studies assessing roadside spring water quality in the Central Appalachian region, consumption of untreated roadside spring water can pose a health risk to spring users due to the presence of total coliform and *E. coli*, among other bacteria and pathogens. However, as at-home water options may be unpalatable and/or spring water consumption may have cultural significance, education dissuading spring water use entirely may be ineffective and poorly received. Bleach disinfection of roadside spring water can provide a simple, accessible POU treatment option for households reliant on roadside spring water. This study demonstrates that 2 drops of household bleach from an eyedropper provided sufficient disinfection and free chlorine residual in 1 gallon of roadside spring water for up to one month. Efforts to provide information on spring water quality and bleach disinfection via infographic were considered helpful but only half of survey respondents reported that they intended to use the bleach disinfection instructions. Additional research on the risks associated with spring water use, as well as efforts to expand water infrastructure and improve in-home piped water quality, is needed to better understand and help reduce associated health risks in Central Appalachia and elsewhere where untreated spring water is used as a source of drinking water.

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Supplementary Figure

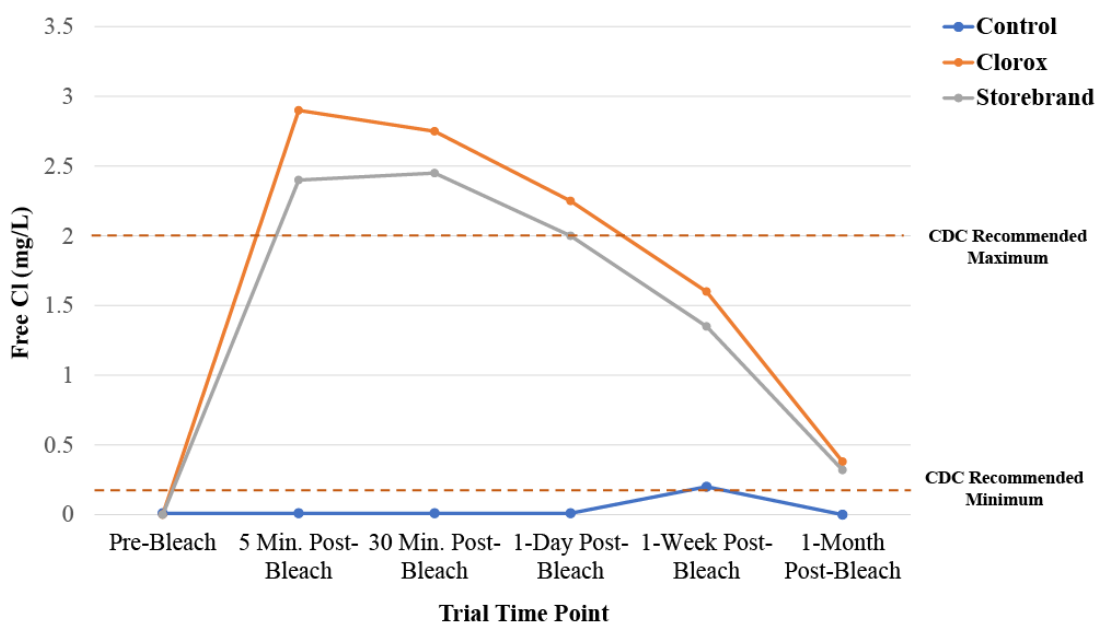


Figure S1. The relationship between bleach brand (2 drops per 1 gallon of spring water) and free chlorine measured in collected spring water over time.