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Occurrence of male-specific and somatic coliphages and relationship with rainfall in privately-owned wells from peri–urban and rural households

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ABSTRACT

Privately-owned drinking water wells serving fewer than 25 people (private wells) are prevalent and understudied across most of the US. Private wells primarily serve rural households located outside of municipal drinking water and sewerage service coverage areas. These wells are not regulated by United States Environmental Protection Agency (EPA) under the Safe Drinking Water Act, are not regularly monitored by any public agency or utility, and generally do not undergo disinfection treatment. Coliphages are a group of viruses that infect coliform bacteria and are useful viral surrogates for fecal contamination in water systems in much the same way that fecal indicator bacteria (FIB), such as *E. coli* and to a lesser extent total coliforms, are used to quantify fecal contamination. Coliphages are approved by the EPA for regulatory monitoring in groundwater wells in the USA, but are not routinely used for this purpose. The present study characterizes the occurrence of male-specific and somatic coliphages, along with FIB, in private wells ($n = 122$) across two different counties in North Carolina. While occurrences of *E. coli* were rare and frequency of total coliform was generally low (~20%), male-specific and somatic coliphages were detectable in 66% and 54% of samples, respectively. Concentrations of male-specific coliphages were higher than somatics at each county and on a monthly basis. Rainfall appears to be partly influencing higher coliphage concentrations in December, January and February. This research underscores the need for increased surveillance in private wells and consideration of using coliphages in order to better characterize occurrence of fecal contamination at the time of sampling, especially during rainier months.

1. Introduction

Microbial contamination from fecal sources compromises the integrity of drinking water systems and threatens the health of consumers. In the USA. alone, 7.2 million waterborne illnesses occur annually from a variety of water sources (Collier et al., 2019). Groundwater sources serve 90.5 million people using community groundwater systems and 48 million people using private wells in the USA. Resilience of these systems to contamination events depends on their construction, design, and operation, as well as on the aquifer's geohydrological, physical, chemical and microbial integrity (Colford et al., 2006; EPA 2015; Griebler and Avramov 2014). From a 36-year (1971–2006) assessment of disease outbreaks from drinking water sources conducted by Craun et al. (2010), untreated, inadequate, or interrupted groundwater was responsible for over half (422 of 801) of water system deficiencies in the 780 waterborne disease outbreaks summarized. Individual private wells and private water systems accounted for 82 of these outbreaks.

Private wells are particularly vulnerable to microbial contamination due to lack of residual disinfection and the absence of monitoring requirements under the Federal Safe Drinking Water Act. While testing is required at the time of initial installation

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and/or property sale in some states, the overall level of testing is low. Homeowners in rural and peri–urban areas are especially at risk of exposure to microbial pathogens because they may be unaware of suggested federal monitoring guidelines and may have limited financial resources or information to address monitoring and treatment issues (Allevi et al., 2013; Gasteyer and Vaswani 2004; Wescoat et al., 2007).

Human enteric viruses have been found in municipal wells (Abbaszadegan et al., 2003; Fout et al., 2017) and have been associated with increased incidence of acute gastrointestinal illness (AGI) within a community (Borchardt et al., 2012). In the above-mentioned 36-yr assessment of outbreaks by Craun et al. (2010), for 55.4% of drinking water systems overall and 67.1% of individual systems, viruses made up 8.2% and 12.2% of the etiological agents respectively (Craun et al., 2010). A viral etiology has also been suggested in many outbreaks of unknown acute gastrointestinal illnesses (Reynolds et al., 2008). According to Brunkard et al. (2011), 22 out of 36 drinking water-related outbreaks in the USA during 2007–2008 were attributed to groundwater sources; 5 of these 22 outbreaks were attributed to viral pathogens, while bacteria accounted for the largest proportion of the groundwater outbreaks (11 of 22).

Direct detection of viral pathogens in drinking water is time consuming and expensive. Water quality stakeholders generally use approved fecal indicator bacteria (FIB) such as total coliforms, *E. coli*, and sometimes coliphages (i.e., viruses that infect coliform bacteria) to indicate presence of fecal contamination and the potential presence of associated pathogens (Dufour 1984; EPA 2001a, b). Total coliforms while not always indicative of fecal pathogens are useful proxies for a structurally or functionally compromised well. While several studies have reported the prevalence of total coliforms and *E.coli* in private wells in the USA, with a wide range of detections for total coliforms (Allevi et al., 2013; Bauder et al., 1991; Borchardt et al., 2003; DeSimone et al., 2009; Kross et al., 1993; Sandhu et al., 1979; Sworobuk et al., 1987), few studies have assessed the occurrence of fecal indicator viruses in either community-based or private groundwater wells (Salter and Durbin 2012). Bacteriophages were found in 20.7% of 448 wells sampled in 35 USA states by Abbaszedegan et al. (2003); however, wells in North Carolina (NC) were not assessed. Coliphages may be preferred indicators of human enteric viruses in groundwater wells (Havelaar et al., 1993; Holcomb and Stewart 2020; Snowdon et al., 1989) due to their small size in comparison to FIB (23–80 nm vs. 0.5–3 μm) and the consequent ease of movement through subsurface soils reported as far down as ~100 m (Keswick et al., 1982).

Few peer-reviewed studies report on fecal indicator viruses in private wells, and the Southeast is particularly poorly studied. Our study focused on rural and peri–urban areas of the southeast, specifically NC. The main goal of this study was to design a monitoring framework to investigate the microbial quality of private wells, using two counties in rural and peri–urban North Carolina representing unique environmental hazards. Specific aims were to: 1) present occurrence and compare frequencies of the two coliphage groups, somatics and male-specifics, with bacterial indicators in the counties individually and combined; 2) compare concentrations between coliphage groups in each and combined counties; and 3) perform monthly comparisons of coliphage concentrations for each coliphage group per county and determine relationships with rainfall totals.

Materials and methods

Site selection and sample collection

Private wells serving single households that use untreated well water for consumption and other domestic activities were selected for sampling in Robeson and Orange Counties, NC (Fig. 1). Households were identified by convenience sampling in coordination with county health departments, other community partners, and by word-of-mouth. Sites were situated within the Lumber River Watershed (Hydrologic Unit Code 08) in Robeson County or within the Haw River Watershed, part of the Cape Fear Basin in Orange County. The Lumber River Watershed drains 12,372 acres and land use/cover consist of 61% agricultural, 32.7% natural, 3.9% cultural in the form of urban and transportation corridors, and 2.4% other (e.g., open water or barren rock). Approximately, 68% of soils in the Lumber Watershed are considered hydric soils which are generally classified as poorly draining. The Haw River Watershed is considerably larger and drains 1526 m^2iles with 27% agricultural, 43% forest, 17% urban, and 13% land use cover categories. Soils in Orange County are either well or moderately-drained.

Wells were sampled at each of the following periods of time: 1) late July/early Aug; 2) October; 3) November; 4) December 2019; 5) January; and 6) February 2020. Repeat monthly sampling occurred for a subset of wells in most months for Orange ($n = 4$) and Robeson ($n = 12$) counties and the remainder of wells in Robeson ($n = 42$) were sampled on a one-time basis in July ($n = 4$), October ($n = 2$), November ($n = 10$), December ($n = 9$), and January ($n = 17$) for a total of 122 samples. Age of wells in years ranged from <20 ($n = 14$), 20–30 ($n = 8$), >30 ($n = 18$) or were otherwise unknown ($n = 18$). Depth of wells in meters were <10.5 ($n = 19$), 10.6–30.5 ($n = 7$), >30.5 m ($n = 7$), or homeowners did not know depth of wells ($n = 25$). All of the households in the sampling campaign are also serviced by a septic system. The inside and outside of well-head spigots (or the nearest outdoor spigot/access point if the wellhead itself did not have a tap) were wiped with 70% ethanol; the ethanol was allowed to evaporate for approximately 30 s and the well-head spigots were then flushed at full flow for one minute before sample collection. Water was collected directly into autoclave-sterilized 4 L polypropylene containers, placed on ice for transport, and refrigerated at 4 °C prior to processing, which generally occurred within 24 h. In some cases, sample hold times were extended to within 30 h for bacterial analysis and 48 h for coliphage assays. Based on guidance from EPA protocols, a 30-hour window is an acceptable processing time for coliform samples collected for non-regulatory purposes. A 48-hour time limit is also acceptable for coliphage analysis according to EPA Method 1602: Male-specific (*F+*) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure (EPA 2001b, 2015). Regardless of when samples were taken, entire monthly precipitation data were retrieved from the nearest United States Geological Survey gaging stages in either Orange (USGS 355,520,079,035,845 Bolin Creek Village Drive) or Robeson County (USGS 02105500 Cape Fear River at William O' Huske Lock).

Sample processing and analysis

Coliphages were enumerated using EPA Method 1602: SAL Procedure (EPA 2001b). Briefly, either the somatic (*E. coli* CN-13; ATCC#700,609) or male-specific (*E. coli* Famp; ATCC#700,891) coliphage host was grown to exponential log phase (confirmed by A_{540} measured using a spectrophotometer). The log-phase host plus 0.5 mL of 4 M $MgCl_2$ was added to 100 mL of sample (held at 36.5 °C in a water bath). The resulting sample + host was then added to 100 mL of molten 2X tryptic soy agar containing either nalidixic acid (200 mg/L) or ampicillin/streptomycin (30 mg/L) as selective antibiotics for the somatic or male-specific hosts, respectively. The 200 mL aliquot of sample-agar mixture was then divided evenly and plated on five replicate 150 mm x 15 mm plates, allowed to solidify, and incubated at 36.5 °C. Plaques, which indicate lysis of the *E. coli* host by infectious coliphages, were counted at 20–24 h, and were reported as plaque forming units per 100 mL

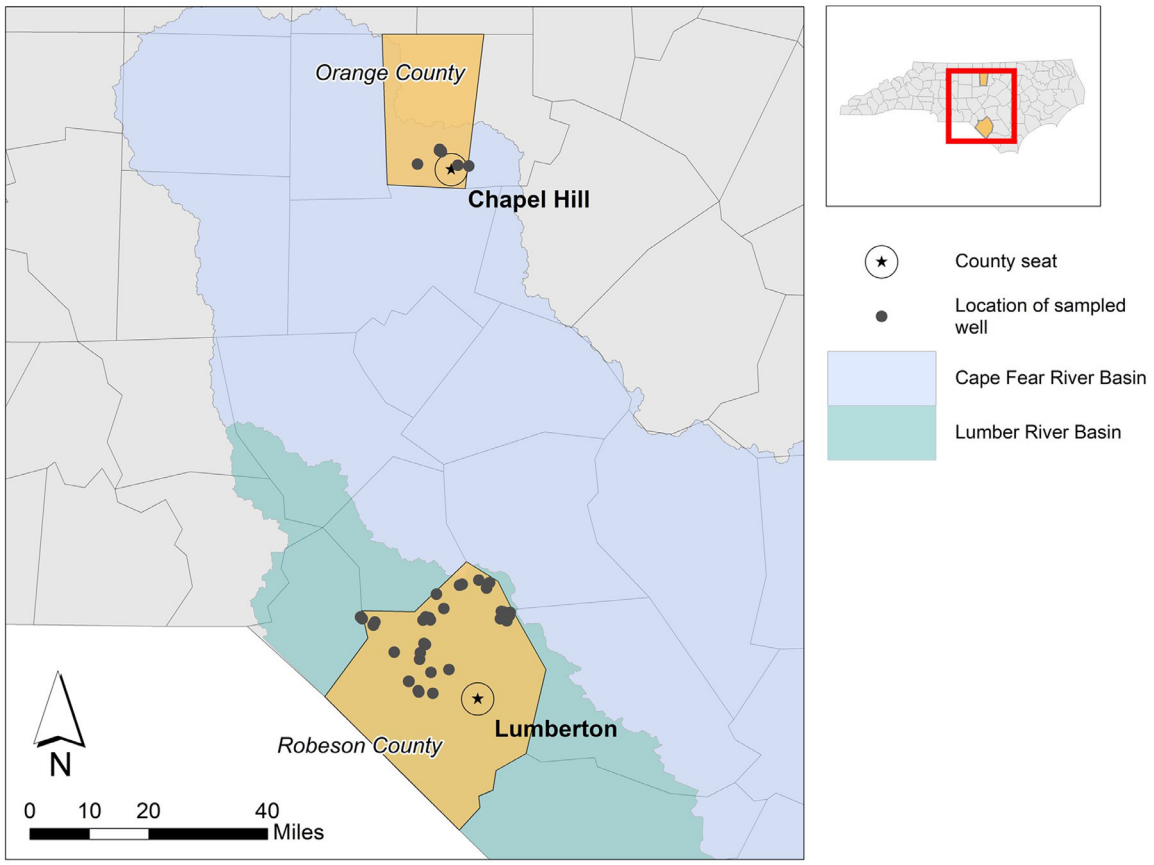

Fig. 1. Sampling locations for privately-owned groundwater wells in Robeson and Orange Counties, North Carolina.

of original sample volume (PFU/100 mL). Ambiguous plaques were confirmed by a spot-plate test per Method 1602.

Bacterial contamination in wells was quantified by EPA Method 1604: Total coliform and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium) (EPA 2002). Because bacterial concentrations were expected to be low in well water, a volume of 1000 mL instead of 100 mL was vacuum-filtered through a sterile mixed cellulose ester 0.45 µm filter (Millipore EMD). Filters were rinsed with phosphate buffered saline solution and transferred to 60 mm x 15 mm culture plates containing MI agar. Plates were incubated at 36.5 °C and colony forming units per 100 mL of sample (CFU/100 mL) were counted after 24 h incubation.

Statistics

A z-score test was used to compare the frequency of occurrence for coliforms (total coliforms, *E. coli*) and the frequency of male-specific, somatic, or either coliphage group for each individual county and the two counties combined. A *t*-test was performed on log-transformed concentrations to compare mean differences between coliphage type within each county and on a combined county basis. Values were initially log transformed to reduce skewness and improve normality. A *t*-test was assessed in advance of pooling a coliphage type within a county for combined county analyses. In order to perform statistical analyses, in the case of samples with non-detect values, one-half of the detection limit of (i.e. 0.05 CFU/100 mL or 0.5 PFU/100 mL) was assigned as a continuity correction for statistical comparisons (EPA 2006; Silvestri et al., 2017). Separate one-way ANOVA tests followed by a post-hoc analysis using Dunnett's T3 were used to compare mean monthly log10 concentrations for each phage type per county. A non-parametric Spearman's rank correlation coefficient was used to determine if a relationship existed between monthly rainfall levels and coliphage concentrations for each phage type per county. An alpha (α) = 0.05 was used as the significance level of all statistical tests. Results were presented as plaque-forming units per 100 ml (PFU/100 ml) for coliphage analysis and colony-forming units per 100 ml (CFU/100 ml) for total coliform and *E. coli* analyses.

Results

Occurrence and frequency comparison of coliphages and coliforms in private wells

Overall, coliphages, regardless of group, were detected more frequently than bacterial indicators in the counties both individually and combined. While *E. coli* was detected only twice (10%) in Orange county, either coliphage group was detected more frequently, at 55%. In Robeson County, *E. coli* was not detected, but either coliphage was detected in 74% of samples (Fig. 2). Either coliphage was detected at 40%, 57%, and 54% more frequently than total coliforms in Orange, Robeson, and the combined counties, respectively (Fig. 2).

Fig. 3

Results of z-score tests comparing frequencies of coliforms and coliphages are presented in Table 1. Observations of *E. coli* in Orange were rare ($n = 2$) and, expectedly, frequencies of occurrence were different than for male-specific, somatic, or either coliphage type ($p < 0.001$). There were significant differences ($p < 0.05$) in the proportions between coliforms and coliphages in most cases, except for total coliforms and somatic coliphages in Orange County ($p = 0.10$) (Table 1).

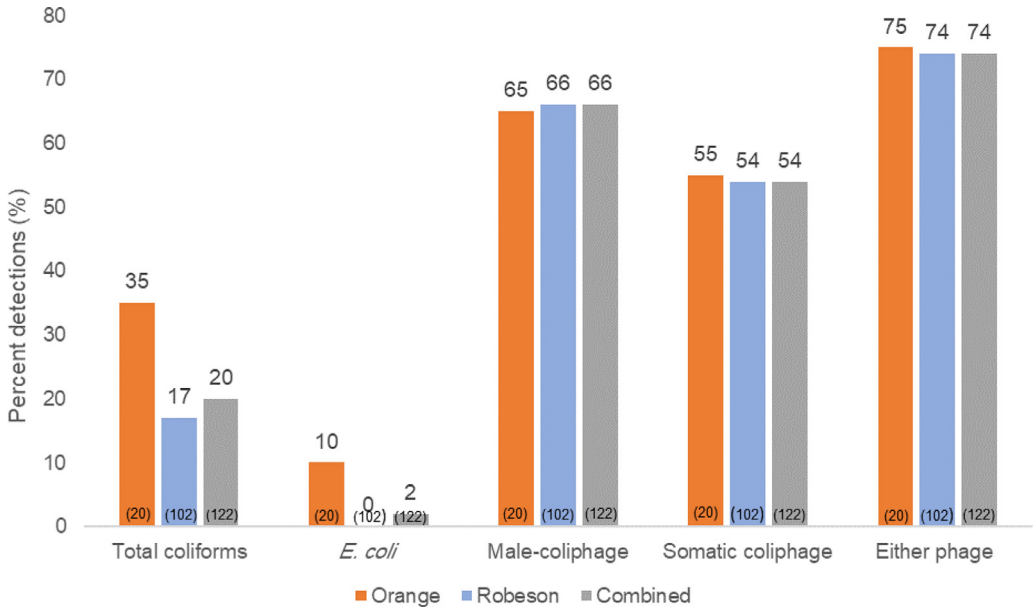

Fig. 2. Percent occurrences for fecal indicator bacteria (total coliforms, *E. coli*) and viruses (male, somatic, or either coliphage) from private well samples in Orange (orange bars) or Robeson County, North Carolina (blue bars) or both counties combined (gray bars). Numbers in parentheses represent number of samples collected.

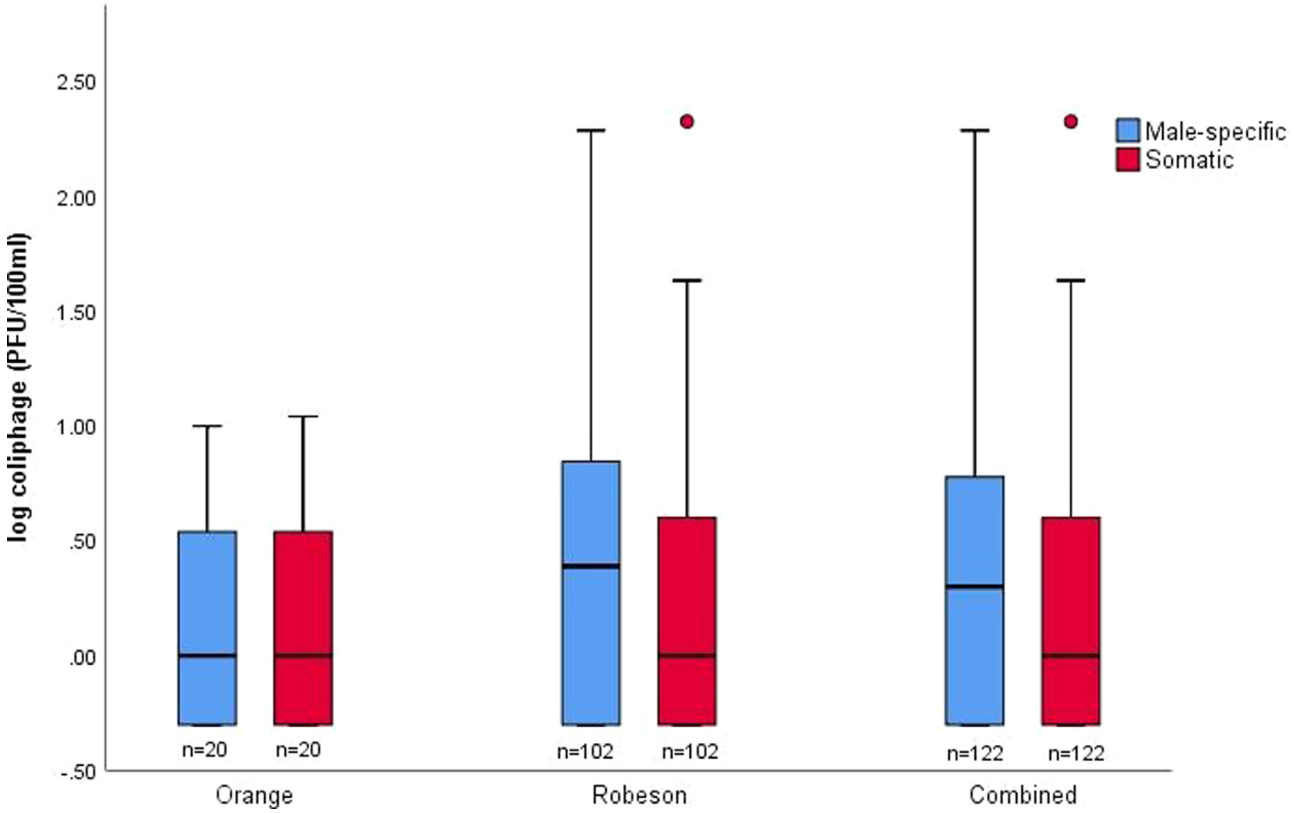

Fig. 3. Comparisons between male-specific and somatic coliphage concentrations ($\log_{10}$ PFU/100 mL) from water samples ($n = 122$) collected from private wells in Orange and Robeson Counties, North Carolina. Asterisk indicates concentrations are significantly different from each other ($p < 0.05$). PFU = plaque forming units

Comparison of concentrations between coliphage type for individual and combined counties

The concentrations of coliphages were generally low in all samples and male-specific (mean $\log_{10}$ of 0.16 PFU/100 mL) and somatic coliphage concentrations (mean $\log_{10}$ of 0.14 PFU/100 mL) were similar to each other in Orange County ($p = 0.888$). However, male-specific coliphage concentrations were higher than somatic concentrations in Robeson (male-specific: mean $\log_{10}$ of 0.41, somatic: mean $\log_{10}$ of 0.19 PFU/100 mL, $p < 0.05$) and combined counties (male-specific: mean $\log_{10}$ of 0.37, somatic: mean $\log_{10}$ of 0.17 PFU/100 mL, $p < 0.05$).

Table 1
Z-score test results for frequency of occurrence comparisons between coliform (total coliforms, E. coli) and male-specific, somatic, or either coliphage type in Orange or Robeson County, North Carolina or counties combined.

	Male-specific coliphages	Somatic coliphages	Either coliphage type
Orange County			
Total coliforms	$z = 1.9$ p=<*0.05*	$z = 1.3$ $p = 0.10$	$z = 2.5$ $p < 0.01$
E. coli	$z = 3.6$ $p < 0.001$	$z = 3.0$ $p < 0.01$	$z = 4.2$ $p < 0.001$
Robeson County			
Total coliforms	$z = 7.1$ $p < 0.001$	$z = 5.6$ $p < 0.001$	$z = 7.8$ $p < 0.001$
Counties combined			
Total coliforms	$z = 8.2$ $p < 0.001$	$z = 5.5$ $p < 0.0001$	$z = 10.9$ $p < 0.001$

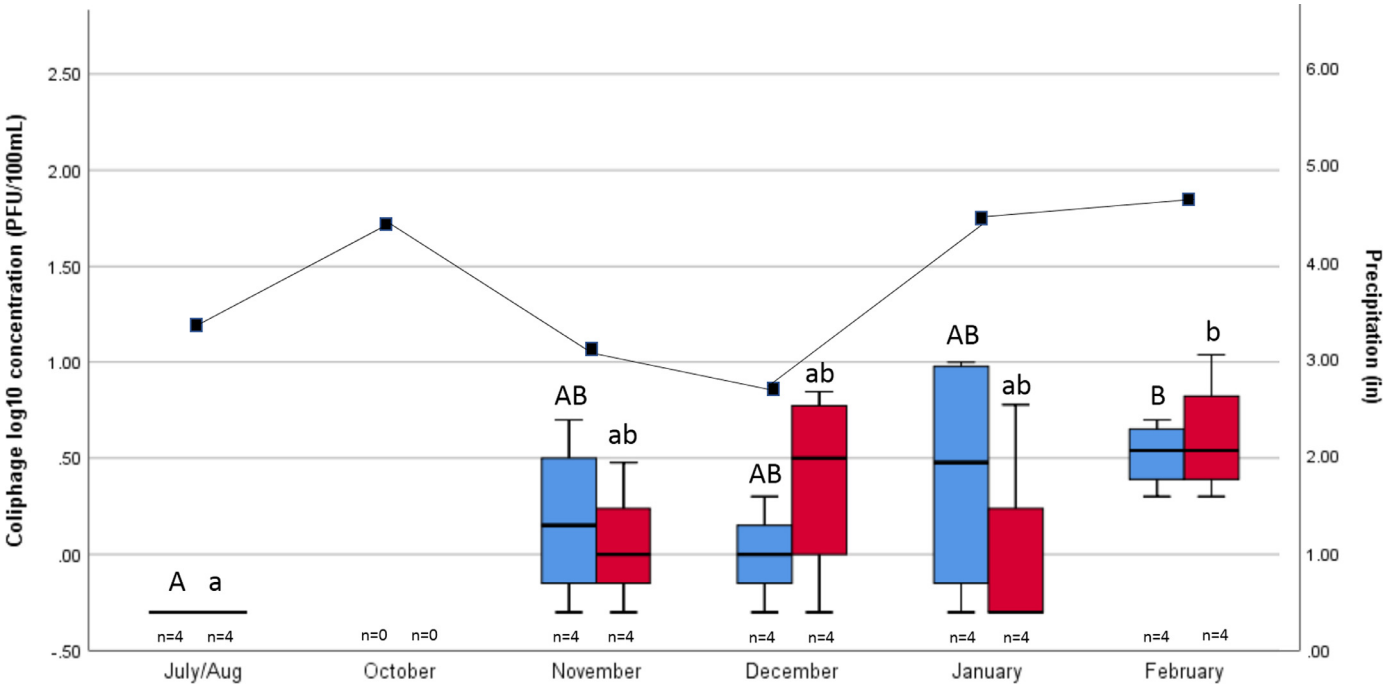

Fig. 4. Comparison of monthly coliphage concentrations (log_{10} PFU/100 ml) for samples collected from private groundwater wells in Orange County, North Carolina. Months sharing the same capital letters for male-specific coliphages (blue boxplots), and lowercase letters for somatic coliphages (red boxplots), are not significantly different from each other ($p > 0.05$). Black line connects monthly rainfall totals (precipitation, inches). Nearest data for monthly total rainfall in Orange County was retrieved from USGS 355,520,079,035,845 Bolin Creek Village Drive rain gage at Chapel Hill, NC. Samples were not collected in October. PFU = plaque forming units

One-way Anova for monthly comparison of coliphage type in each county

In Orange County, monthly mean log_{10} concentrations differed significantly for either male-specific ($F_{4,15} = 3.034$, $p = 0.05$) or somatic coliphages ($F_{4,15} = 3.369$, $p < 0.05$) (Fig. 4). Post-hoc analyses revealed only February log_{10} concentrations were significantly different, being 0.8 of a log_{10} and 0.9 of a log_{10} higher than July/Aug for male-specific ($p < 0.05$) and somatic ($p = 0.05$) coliphages, respectively. All other monthly concentrations were similar (Fig. 4). In Robeson County, monthly mean log_{10} concentrations differed significantly for either male-specific ($F_{5,96} = 13.402$, $p = 0.001$) or somatic coliphages ($F_{5,96} = 6.262$, $p < 0.001$) (Fig. 5). For male-specific coliphages, October and November log_{10} concentrations ranged between 0.6 and 1.1 of a log_{10} less than other months while July/Aug, December, January and February were similar to each other ($p < 0.05$; Fig. 5). Monthly differences for somatic coliphages in Robeson were more variable, with July, December, January, and February concentrations not significantly different from each other, and July/Aug, October, and November not significantly different from one another (Fig. 5). For the subset of repeated samples in Robeson, significant differences occurred both in the case for male-specific ($F_{5,54} = 6.659$, $p < 0.001$) and somatic coliphages ($F_{5,54} = 6.659$, $p < 0.001$). The significance patterns of the repeated sample subset for male-specific coliphage concentrations were exactly the same as results presented in Fig. 5 inclusive of repeat and one-time samples (data not shown). Differences in somatic coliphage concentrations in subset of repeated well samples were overall similar to the entire dataset except for the lack of statistical differences between July and February and for November and December (data not shown). Repeat samples do not appear to confound the variability of either male-specific or somatic coliphages concentrations.

Spearman rank correlation test for association between monthly rainfall totals and concentration for each coliphage and county

No relationship was found between rainfall and either somatic ($p = 0.756$) or male-specific coliphages ($p = 0.103$) in Orange County. On the other hand, a moderate positive relationship was found between somatic coliphages ($r = 0.440$, $p < 0.001$), as well as male-specific coliphages ($r = 0.562$, $p < 0.001$), and monthly rainfall in Robeson County.

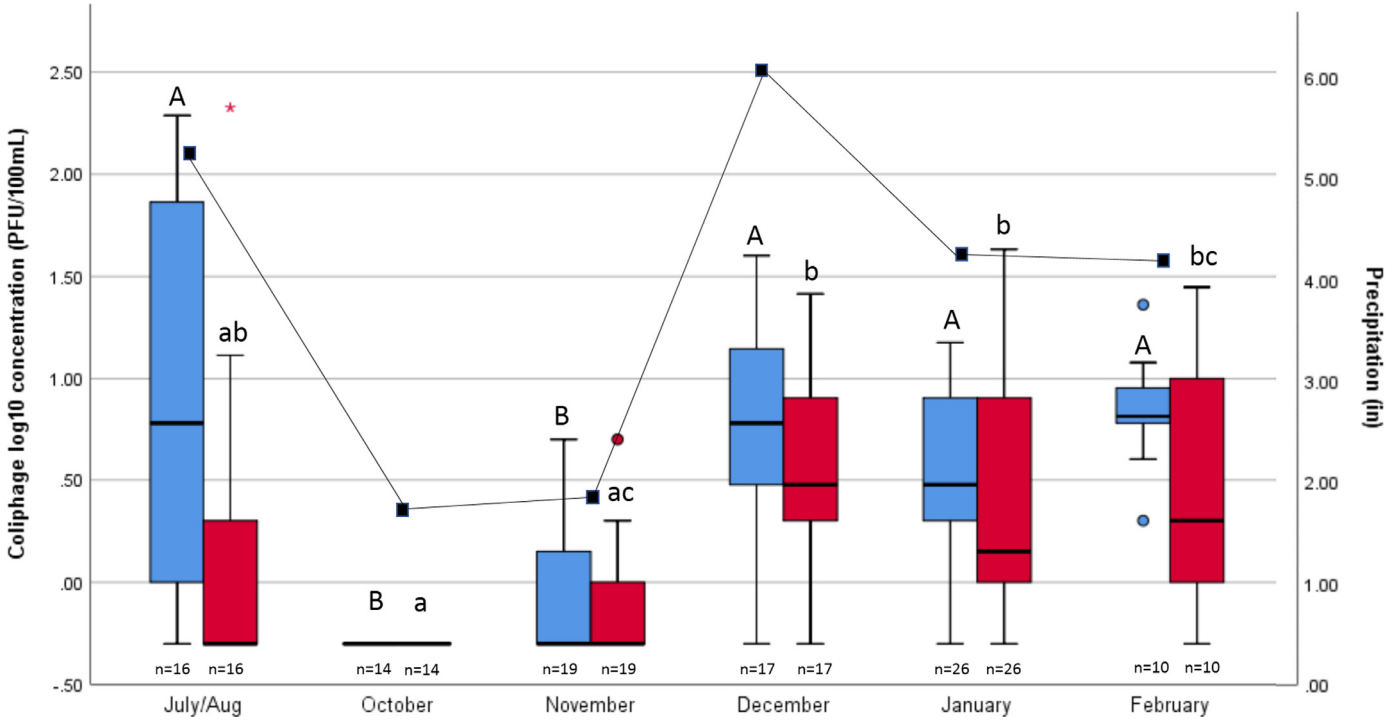

Fig. 5. Comparison of monthly coliphage concentrations (log_{10} PFU/100 ml) for samples collected from private groundwater wells in Robeson County, North Carolina. Months sharing the same capital letters for male-specific coliphages (blue boxplots), and lowercase letters for somatic coliphages (red boxplots) are not significantly different from each ($p > 0.05$). Black line connects monthly rainfall totals (precipitation, inches). Nearest data for monthly total rainfall in Robeson County was retrieved USGS 02,105,500 Cape Fear River at William O' Huske Lock, Tarheel, NC rain gage. PFU = plaque forming units

Discussion

Coliphage occurrence and comparison to FIB

Studies have often reported on the occurrence of enteroviruses and other viruses in groundwater systems, both private and public wells, but there are limited studies performed on coliphage detection in private wells. Including coliphage data for private wells is of particular interest because fecal viral contamination of public health concern may be present in such sources even when a particular human pathogenic virus may not be detected in a given sample. The frequency of detection in the present study is considerably higher than in previous research conducted by EPA on groundwater wells specifically from the southeast, including NC, and other regions in the United States (EPA 2006). Researchers reported that somatic coliphages were found in 7% of the wells (2 of 27 wells) and male-specific coliphages were found in 4% of the wells (1 of 27 wells) in the southeastern USA, while somatic and male-specific coliphages were detected in 57% (16/28) and 39% (11/28) of wells in the Northeast. The prevalence reported in the present study was broadly comparable to the prevalence of coliphages detected in wells from the northeastern USA. Differences between the present study and the 2006 EPA study, as well as between regions in the EPA study, may be explained in part by differences in the types, depths, and/or construction characteristics of wells sampled, sampling months, temperature, and/or rainfall, and proximity to septic systems or other environmental sources of fecal contamination. All of the wells in the present study were private wells sampled in the sampling window from July/August to February, whereas the samples in the EPA study included public water groundwater supply and non-community transient public water supplies (campgrounds) but sampling months were not reported.

Male-specific, somatic, or either coliphage was detected in greater proportions than total coliforms or *E. coli* in the present study. *E. coli* was found in two cases in Orange County only and was detected at very low levels (1 CFU/1000 mL). The occurrence of total coliforms in 20% of water samples from private wells in this study is roughly comparable to results reported for coliforms in private well water samples from Wake County, NC (Stillo and Gibson 2017), as well as studies reporting occurrence of total coliforms in samples from wells in Iowa (27%) and Nebraska (26%) (Gosselin et al., 1997; Kross et al., 1993). Most FIB do not always have direct relationship with coliphage or pathogenic viruses and do not always predict health risks (Leclerc et al., 2000; Noble and Fuhrman 2001; Payment and Locas 2011); however, coliphages do appear to have a positive association with viral pathogens (Vergara et al., 2015). Given this potential association and the high coliphage prevalence in private wells in this study, incorporating a multi-indicator approach may be more informative about the microbiological quality of groundwater (Lucena et al., 2006) and using coliphages in addition to or in place of FIB is suggested.

Occurrence and concentration of coliphage type in each and combined counties

Studies have overall reported somatic coliphages in greater proportions than male-specific coliphages in surface water (Nappier et al., 2019), but this trend is not always seen in groundwater systems (Jofre et al., 2016). In the present study, it is interesting to note the frequencies of male-specific coliphages were only about 10% higher than somatic coliphages, but male-specific concentrations were 0.4 of a log_{10} higher in Robeson and combined counties. Similarly, the pattern of higher levels of male-specific to somatic phages emerged on a monthly basis in most cases. Researchers have found soil characteristics and attachment affinities

of phage groups could play a role in their differing occurrence in groundwater (Jofre et al., 2016; Skraber et al., 2007); others suggest differential susceptibilities of different bacteriophage families to unfavorable environmental factors such as high or low pH, high salinity, etc. (Jończyk et al., 2011). In the present study, the concentration range for either coliphage type was greater in Robeson than Orange County and soil type and land use could play a role in this observation. Approximately 68% of soils in Robeson County are classified as hydric soils with poor drainage characteristics that could promote coliphage persistence in ponded areas surrounding unprotected wellheads after rainfall events with eventual intrusion and infiltration into especially shallow wells. Agriculture is the dominant land use in Robeson County and deposition of coliphages in fecal waste from livestock may contribute to the greater range of coliphages than in Orange County. Assessment of hydrogeological and physicochemical properties that may be influencing differential phage type occurrences and concentrations is warranted.

The concentrations for male-specific coliphages in this study are similar to baselines levels (10 to 30 PFU/100 mL) in a study investigating gastrointestinal illness in users of an artificial whitewater course (Lee et al., 1997), while somatic coliphage concentrations are comparable to levels (0.3 to 1.7 PFU/100 mL) found at a marine beach that were associated with GI illness in bathers (Abdelzaher et al., 2011, Abdelzaher et al. 2010). Although these studies are not groundwater related, this does provide evidence to further consider future epidemiological studies associated with coliphages in groundwater wells.

Monthly evaluation of coliphage type in each county and relation to rainfall

Concentrations for phages were generally higher in July/Aug, December, January and February than October and November, at least for Robeson County wells.

There was a positive relationship for both phage groups and monthly rainfall amounts, which suggests precipitation, in part, as a factor driving coliphage concentrations. The pattern was less evident in Orange County and could have been because of less variability of rainfall or small sample sizes reducing the effect size. Studies investigating monthly profiles of coliphages in groundwater are limited, but one study from USA groundwater wells found peaks in July and November for a male-specific phage (Abbaszadegan et al., 2003). Male-specific phages were 137 PFU/100 mL in July from a community well water system serviced by a groundwater source and dropped to 7 PFU/100 mL by September (Atherholt et al., 2003). Nappier et al. (2019) conducted a meta-analysis of coliphage occurrence in wastewater and surface water and reported higher coliphage concentrations in the months of December through May than June through November. Our findings are partially consistent with the findings of Nappier et al. except for the peak in July/Aug in Robeson County which may have been elevated after a rain event. When homeowners provided well depth, over half (57%) wells were less than 10.6 m or 35 ft and transport of phages during rainfall events could have impacted shallow wells more so than deeper ones. Sampling coverage of warmer and rainier months, such as March, April, and May, will be important to gain a picture of how temperature during wetter season factor into coliphage presence.

Measurement of viral pathogens (Noble et al., 2003) and serotyping of *F+* coliphages to discriminate between human and non-human sources of fecal contamination (Brion et al., 2002; Cole et al., 2003; Griffin et al., 2000; Stewart-Pullaro et al. 2006) to elucidate sources of coliphage is warranted. All of the households in the sampling campaign are also serviced by a septic system and it is plausible sewage could have infiltrated into wells during rainfall events. Going forward, use of sophisticated Bayesian Maximum Entropy (BME) mapping analysis to describe spatial distribution of microbial contamination and land use regression to identify key spatial determinants, such as rainfall, flooding, proximity to animal feeding operations, soil characteristics, and integrity of septic and sewage systems, that could influence coliphage presence will lead to a more holistic understanding of resilience of private wells and risk to homeowners.

Conclusions

The frequency of detection of coliphages was considerably higher than in previous research conducted on groundwater wells from the southeastern USA. Male-specific, somatic, or either phage was detected in greater proportions than total coliforms or *E. coli* in both Robeson and Orange Counties. Frequencies and concentrations of male-specific coliphages were higher than of somatic coliphages and the pattern was evident on monthly basis in most cases. Rainfall appears to be partly influencing higher coliphage levels in July/Aug, December, January and February. This work underscores the utility and importance of considering coliphages, in conjunction or instead of FIB, to investigate contamination in private drinking wells to protect consumers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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