Associations between fecal indicator bacteria prevalence and demographic data in private water supplies in Virginia

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ABSTRACT

Over 1.7 million Virginians rely on private water sources to provide household water. The heaviest reliance on these systems occurs in rural areas, which are often underserved with respect to available financial resources and access to environmental health education. This study aimed to identify potential associations between concentrations of fecal indicator bacteria (FIB) (coliforms, *Escherichia coli*) in over 800 samples collected at the point-of-use from homes with private water supply systems and homeowner-provided demographic data (household income and education). Of the 828 samples tested, 349 (42%) of samples tested positive for total coliform and 55 (6.6%) tested positive for *E. coli*. Source tracking efforts targeting optical brightener concentrations via fluorometry and the presence of a human-specific *Bacteroides* marker via quantitative real-time polymerase chain reaction (qPCR) suggest possible contamination from human septage in over 20 samples. Statistical methods implied that household income has an association with the proportion of samples positive for total coliform, though the relationship between education level and FIB is less clear. Further exploration of links between demographic data and private water quality will be helpful in building effective strategies to improve rural drinking water quality.

Key words | coliform bacteria, *E. coli*, private water system, source tracking, water quality

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INTRODUCTION

Estimates from various government agencies report that 23 to 45 million Americans currently rely on private water supply systems (wells, springs, and/or cisterns) for drinking water (USGS 2009; USEPA 2013). The United States Environmental Protection Agency (USEPA) regulates public drinking water systems (e.g., systems serving more than 15 connections or 25 individuals at least 60 days each year) via the Safe Drinking Water Act (SDWA) of 1974, which details health-based water quality standards and specific monitoring requirements. Since the implementation of the SDWA and related supplemental statutes, the proportion of the national population served by water systems that meet all health-based standards has steadily increased (USEPA 1999). However, the SDWA does not apply to private water supply systems; the USEPA simply provides guidance to homeowners with private water supply systems that emphasizes annual testing and routine maintenance (USEPA 2013). Ongoing reports documenting the incidence of coliform bacteria contamination in private wells suggest that these recommendations are not being communicated to homeowners, are being ignored, or are simply insufficient to protect public health (Bauder *et al.* 1993; Kross *et al.* 1993; Gosselin *et al.* 1997; Allevi *et al.* 2013).

The US Centers for Disease Control recently stated that the number of reported infectious waterborne outbreaks has increased among populations served by small community or private drinking water systems (Craun *et al.* 2010). Systems relying on groundwater are of particular concern, as increasing anthropogenic influence, even in relatively rural areas, has rendered aquifer contamination with pathogenic

doi: 10.2166/wh.2014.026

microorganisms fairly ubiquitous (Macler & Merkle 2000). A recent US Geological Survey (USGS) study of over 1,389 private household wells sampled at the point-of-entry reported that 23% of samples exceeded at least one of the SDWA's maximum contaminant levels (MCLs) for chemical contaminants (e.g., nitrate, fluoride, pesticides). In addition, 34% of samples were positive for total coliforms and 8% tested positive for *Escherichia coli* (USGS 2009). Although this USGS effort aimed to characterize ambient groundwater quality, the reported prevalence of microbial contamination was very similar to that observed by studies examining private water system quality at the point-of-use (POU) (i.e., a faucet within the household). A recent compilation of research and extension studies conducted over the past 35 years indicated that total coliform contamination in samples collected from the POU of private water supplies throughout the United States is not uncommon, with overall incidence ranging from 15 to 85% (Allevi *et al.* 2013).

Several previous surveys of water quality from private drinking water supplies have linked system construction and/or environmental factors to contaminant presence. Bauder *et al.* (1993) and Gosselin *et al.* (1997) explored nitrate and bacterial contamination in private water supply systems in Montana and Nebraska, respectively, and observed that contamination in groundwater varies by geographic region, climatic conditions, underlying geology, and land-use practices. Several survey-based studies have suggested that nitrate and/or bacteria contamination is more common in older, shallower wells (Wojcik *et al.* 1996; Gelberg *et al.* 1999; Allevi *et al.* 2013; Swistock *et al.* 2013).

In contrast, attempts to explore possible connections between observations of water quality in private drinking water supply systems and demographic factors are relatively limited. The only relevant work identified is a 30-year-old study commissioned by the USEPA to gather information about rural water supplies in the USA (Francis 1981). Of the 2,654 samples tested between May 1978 and January 1979, 42% were positive for total coliform and 12.2% were positive for fecal coliform. The study found that households with incomes under $10,000 (N.B. national annual median income in 1978 = $13,512) and households with 'less education' (i.e., no high school degree or equivalent) tended to have 'coliform problems'. No strong association between household demographic data, water supply system, or system characteristics and total coliform contamination was identified. Since the United States Census Bureau has not captured data specific to private water supply systems since 1990, publicly available statistics related to the prevalence and use of private water supply systems relative to home location and family demographics are scarce and likely outdated.

The presence of fecal indicator bacteria (FIB), including coliforms and *E. coli*, suggests the potential presence of enteric pathogens and related human health risk, as these microorganisms are commonly found in mammalian intestines (Savichtcheva & Okabe 2006). However, while the presence of FIB suggests fecal contamination, simple detection does not generally provide information on the origin of this contamination, which can be useful when designing remediation efforts. Several fecal source tracking techniques have evolved in recent decades to assist water quality managers in identifying primary sources of contamination via microbial (e.g., specific species or gene) or chemical (e.g., anthropogenic compounds) markers specific to a given type of waste (Santo Domingo *et al.* 2007). The majority of source tracking applications examine ambient surface waters in combination with large-scale watershed remediation planning (Peeler *et al.* 2006); the application of chemical and microbial source tracking to drinking water quality monitoring has been limited to a handful of previous studies. Allevi *et al.* (2013) investigated 538 samples taken at the POU from Virginia households dependent on private water supply systems for contamination by human sewage. Three of the 538 samples were identified as likely contaminated with wastewater due to high concentrations of optical brighteners, and one sample was positive for a general *Bacteroides* marker (GenBac). A study in Reno, Nevada used the presence of caffeine to identify probable human wastewater contamination within 17 groundwater wells (Seiler *et al.* 2005). Caffeine concentrations as high as 0.23 μg/L were observed in some samples, though this level is considerably lower than concentrations typically found in wastewater (between 20 and 300 μg/L) and septic tank effluents (100–120 μg/L).

The overall goal of the present study is to identify potential associations between bacteria contamination from samples collected from the POU of private drinking water supplies and homeowner-reported demographic

information. In addition to indicator bacteria detection, this study also uses microbial (quantitative real-time polymerase chain reaction (qPCR) detection of the human-specific HF183 *Bacteroides* sequence) and chemical (optical brightener detection via fluorometry) source tracking methods to identify possible sources of contamination to aid in the design of interventions to reduce human risk. A more thorough understanding of the interactions between socioeconomic factors and water quality at the POU will be useful in identifying and supporting appropriate and effective strategies (e.g., educational efforts, subsidized testing or redesigning private water infrastructure) to improve homeowner maintenance of private water supply systems.

METHODS

Sample processing

Samples analyzed in this study were obtained through collaboration with the Virginia Household Water Quality Program (VAHWQP; www.wellwater.bse.vt.edu). Started in 1989, VAHWQP is a Virginia Cooperative Extension effort that aims to improve the water quality and health of Virginians using private water supplies. The VAHWQP organizes county-based drinking water clinics that provide low-cost water quality testing (~$49) to homeowners along with relevant education on system maintenance, potential contamination vulnerabilities, and water treatment options should quality issues be identified.

Drinking water clinics are organized in collaboration with Cooperative Extension county offices and agents. Each clinic consists of four phases: (1) an informational meeting where sample kits are distributed; (2) sample collection on a pre-arranged day; (3) sample analyses; and (4) a final 'interpretation meeting' led by a local extension agent or an on-campus Virginia Tech faculty member who travels to the county where the clinic was conducted. At the interpretation meeting, sample results are returned to participants in sealed envelopes. Each sample kit distributed to participants contains: (1) instructions on how to properly collect the sample; (2) a survey requesting information about the homeowners' perceptions about water quality, potential sources of local contamination, and demographic data; and (3) four pre-sterilized sample bottles. Water samples from each home are tested for 14 separate chemical and bacteriological constituents, including total coliform bacteria, *E. coli*, pH, total dissolved solids, sodium, nitrate-N, sulfate, fluoride, manganese, iron, arsenic, copper, lead, and hardness.

Participants are instructed to collect their sample at the POU (the instructions suggest a non-swivel faucet) and transport them on ice to a central location (often the local extension office) on a pre-arranged sample collection day. All samples are then transported on ice to the Virginia Tech main campus in Blacksburg, VA for analysis. Holding times vary based on the location of the targeted county relative to Blacksburg, but even in the most extreme case, no more than 12 hours pass from the time the samples are collected by the homeowner to when they are analyzed for bacteria. Counties participating in VAHWQP during any given year are selected based on local interest. During 2012, over 800 households in 33 counties participated in sponsored drinking water clinics (Figure 1).

Survey data

Each VAHWQP sample kit includes a survey designed to collect selected household demographic characteristics, including the average household income, the level of education and age of each household member and whether or not each person in the household consumes the water supplied by the private water supply system. Previous research exploring relationships between household water quality and private water supply system design characteristics is available in Allevi *et al.* (2013).

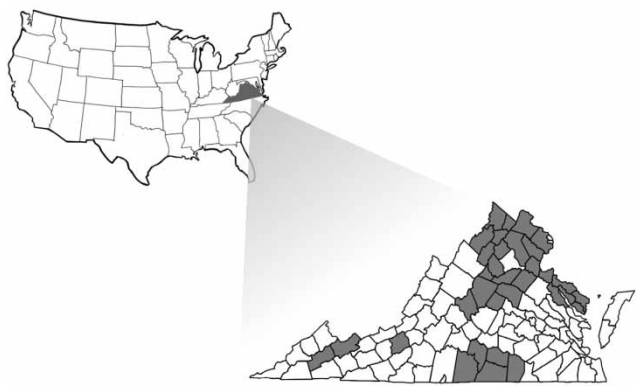

Figure 1 | Counties where VAHWQP drinking water clinics were held in 2013.

Microbial analysis

Following arrival at the Biological Systems Engineering Water Quality laboratory at Virginia Tech, the 120 mL sample bottle from each submitted sample is analyzed for total coliform bacteria and *E. coli* using the Colilert® defined-substrate technique (www.idexx.com, Westbrook, ME, USA). As the Colilert® analysis requires 100 mL of analyte, after inverting the bottle several times to ensure a homogenous mix, excess water (i.e., above the 100 mL fill line) is poured into a 15 mL sterile centrifuge tube and retained for subsequent fluorometry analysis. Quanti-tray®/2000s (capable of detecting counts of up to 2,419 MPN/100 mL) were used to quantify bacteria concentrations. Once filled, each Quanti-tray® was sealed and incubated at $35 \pm 0.5\ ^{\circ}C$ for 24 ± 2 hours. A positive result for total coliform is indicated by a yellow color change and *E. coli*-positive wells fluoresce under ultra-violet light. The number of positive wells is converted to a most probable number (MPN) concentration (MPN/100 mL) based on the Thomas equation (Hurley & Roscoe 1983).

As mentioned previously, a dozen additional water quality analyses are conducted on these samples in conjunction with the VAHWQP drinking water clinics; however, given this study's focus on bacterial contamination, discussion of these results is limited. Previous research by Allevi *et al.* (2013) found no statistically significant associations between bacterial concentrations and phsyio-chemical measures.

Source tracking analyses

All 828 samples collected by the VAHWQP during 2012 were analyzed using a 10AU™ Field and Laboratory fluorometer (www.turnerdesigns.com, Sunnyvale, CA, USA) to detect optical brighteners. Following the collection of samples as described previously, centrifuge tubes were stored in a dark $4\ ^{\circ}C$ refrigerator to prevent UV light exposure, which can degrade anthropogenic optical brighteners (e.g., laundry detergents, toilet paper). At the time of analysis, each sample was transferred to a small cuvette, inverted to re-suspend any particulate matter, and then read by the fluorometer. Readings were recorded following reading stabilization (between 15 and 30 s), and were considered positive if the reading was higher than 30 fluorometric units (Hartel *et al.* 2007; Cao *et al.* 2009). In order to confirm that the reading reflected the presence of anthropogenic optical brighteners rather than naturally fluorescing compounds found in the environment (e.g., dissolved organic matter), each positive sample was exposed to UV light for approximately 4 hours following the initial fluorometry reading. Because anthropogenic optical brighteners are expected to degrade while natural optical brighteners remain stable, the presence of anthropogenic brighteners was confirmed if the fluorometer reading after UV exposure had decreased by 30% or more relative to the original reading (Hartel *et al.* 2007).

Due to the time and expense associated with qPCR, only *E. coli*-positive samples (i.e., those that do not meet current municipal drinking water standards), which were assumed to be most likely to be contaminated with human septage, were analyzed for the presence of the human-specific marker HF183 *Bacteroides*. For the *E. coli*-positive samples, 250 mL of sample water was concentrated via passage through a sterile 0.4 μm Isopore™ membrane filter (www.millipore.com, Billerica, MA, USA) in a sterile biosafety cabinet within 24–72 hours of arrival. Filters were stored in 2 mL cryogenic vials (www.sigmaaldrich.com, St Louis, MO, USA) at $-80\ ^{\circ}C$ until DNA extraction and qPCR analysis was performed (completed within 6 months).

Two microbial source tracking targets were detected via quantitative real-time PCR (qPCR): the general *Bacteriodales* marker (GenBac or AllBac) and human-specific HF183 *Bacteroides*. These fecal indicators were specifically selected as GenBac is used to indicate that mammalian fecal contamination is present and HF183 is used to detect human fecal contamination. Both GenBac and HF183 primers and probes were purchased through Applied Biosystems™ (www.appliedbiosystems.com, Foster City, CA, USA) and are in keeping with those previously published by Seurinck *et al.* (2005) (Table 1). Extraction of DNA was conducted using a QIAamp® DNA Stool Mini Kit (www.qiagen.com, Valencia, CA, USA) according to the instructions provided in the QIAamp® DNA Stool Handbook. Extracted DNA was mixed with 3.0 μL PCR-grade water, 10.0 μL KAPA PROBE FAST qPCR Master Mix, 1.0 μL forward primer, 1.0 μL reverse primer, 4.0 μL probe, 1.0 μL template DNA, and 0.4 μL ROX dye within the qPCR reaction tubes. The thermocycling protocol to facilitate the PCR reaction was conducted using an Eppendorf® Mastercycler® Pro

Table 1 | Primer and probe identities, target genes, and sequences for fecal markers DNA assays

Primer/Probe	Target	Sequence (5′-3′)
GenBac3-F	General *Bacteriodales*	GGGGTTCTGAGAGGAAGGT
GenBac3-R		CCGTCATCCTTCACGCTACT
GenBac3-P		6FAMCAATATTCCTCACTGCTGCCTCCCGTATAMRA
HF183-F	Human-specific *Bacteriodales*	ATCATGAGTTCACATGTCCG
HF183-R		CGTAGGAGTTTGGACCGTGT
HF183-P		6FAMTATCGAAAATCTCACGGATTAACTCTTGTGTACGCTAMRA

-F indicates a forward primer; -R indicates a reverse primer; -P indicates a probe.

(www.ependorf.com, Hamburg, Germany) and is provided in Table 2. All assays also included the following controls: (1) a negative control containing all reagents except the template DNA to detect contaminating amplicons or non-specific amplification; (2) a 'no enzyme' control which omits the reverse transcriptase to confirm that amplification was derived from the synthesized cDNA and not genomic DNA or amplicon contamination; (3) an external positive control consisting of the control template to detect inhibition or a failed reaction; and (4) an internal positive control (ATCC strain of *Enterococcus faecalis*) easily distinguished from the target of interest to pinpoint problems that are intrinsic to the sample reaction. Results were not considered valid if any control deviated from the expected result. Samples were considered negative if the fluorescence was not detectable within 42 amplification cycles.

Statistical analysis

Water quality data and survey results were entered into an Access Database, organized in Excel, and then analyzed in SAS JMP Pro 10.0 (SAS Inc., Cary, NC, USA). The broad goal of the statistical analyses was to identify potential associations between the demographic data obtained via the homeowner survey and the presence/concentration of total coliform and/or *E. coli* in corresponding water samples. Initial attempts to account for interrelationships between various potential factors (e.g., county location, month of collection, presence of sewage and/or animal agriculture) and household demographic characteristics via traditional logistic regression resulted in unstable models, due to the large number of potential input variables relative to a very zero-heavy dataset (i.e., majority of responses were negative). Given this limitation, as well as a skewed population, the simplest available statistical tests were applied, with the goal to identify preliminary potential associations which could be used to motivate future studies targeted at specific communities of interest. The bacteria dataset was tested for normality by comparing a histogram of the data to a normal probability curve, but since it was not normally distributed, non-parametric tests were used to determine associations. Significance was set at an alpha of 0.05.

It must be noted that for statistical analysis, each household was assigned an education level based on responses entered by the presumed 'head of household'. For the purposes of this study, the personal information (e.g., education, age) that was entered first was assumed to correspond to the head of the household. If a child's information was listed first, then the last person's information was assumed to be the head of household.

Table 2 | qPCR cycling protocol

Step	Temperature	Duration	Cycles
Enzyme activation	95 °C	3 min	Hold
Denature	95 °C	3 sec	40
Anneal	60 °C	20 sec	
Extension	72 °C	8 sec	

RESULTS

Participant profile

The 2012 VAHWQP Drinking Water Clinics collected samples from 828 households with an average of 2.3

people per household (1,936 individuals). Questions pertaining to race were not included in the drinking water clinic sample surveys until halfway through the year, so racial information is only available for 927 individuals. A summary of household and participant demographic data is provided in Table 3. Over 30% of the households participating in the 2012 drinking water clinics were Caucasian families with an annual income of over $65,000 (N.B. median Virginian income = $63,302; US Census Bureau 2011), with a head of household that held at least a Bachelor's degree. Although the average participant profile did not necessarily indicate financial or educational disadvantage, almost half of the participating households (48%) had 'never' previously tested their drinking water quality, though the majority of household members (82%) did regularly drink their water.

Microbial contamination

Forty-two percent ($n = 349$) of the 828 samples collected in 2012 were positive for total coliforms, and 7% ($n = 55$) samples tested positive for *E. coli*. Cumulative distribution plots illustrating the full range of observations of bacterial concentration are provided in Figure 2. The observed arithmetic mean total coliform concentration observed was 155 MPN/100 mL, and the mean observed *E. coli* concentration was 12 MPN/100 mL. Twenty-six samples exceeded

Table 3 | Demographic profile of participating households

Sample demographics		
Total number of household members		1,930
Number of households		828
Average number of participants per household		2.3
Number of households' members who drink system water		1,585 (82%)
Racial profile (n = 927 individuals)	White/Caucasian	795 (86%)
	African American	60 (7%)
	Asian American	9 (<1%)
	Hispanic	37 (4%)
	Native American	16 (1%)
	Multi-racial	10 (1%)
Age (n = 1,919 individuals)	<1	8 (<1%)
	1–10	148 (8%)
	10–20	201 (10%)
	21–40	222 (12%)
	41–60	614 (32%)
	61 +	726 (38%)
Levels of income (n = 722 households)	Less than $10,000	26 (3.6%)
	$11,000–$24,000	59 (8.17%)
	$25,000–$40,000	124 (17.2%)
	$41,000–$64,000	134 (18.6%)
	$65,000 or above	379 (52.5%)
Level of education (n = 828, heads of household)	In school now	11 (1%)
	Some high school	25 (3%)
	High school graduate	124 (15%)
	Some college	196 (24%)
	College graduate	209 (25%)
	Post-college (MS, PhD)	221 (27%)
Water tested (n = 818 households)	Never before	389 (47.6%)
	Once before	319 (39%)
	When I think there is a problem	33 (4%)
	Every 5 years	33 (4%)
	Every other year	16 (1.9%)
	Every year	28 (3.4%)

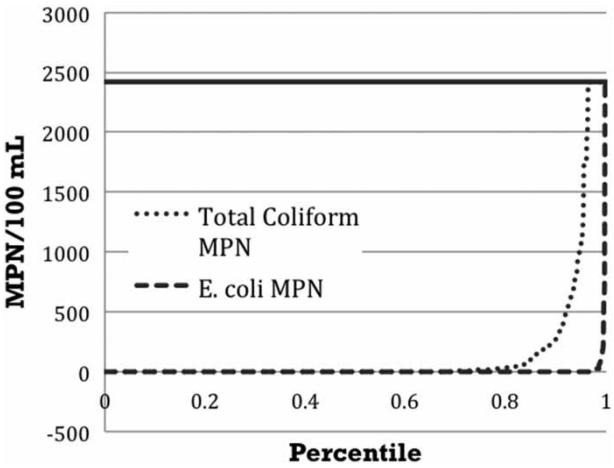

Figure 2 | Cumulative distribution plot for indicator bacteria concentrations. Samples are arranged from lowest concentration to highest concentration on the y-axis and 0 to 1 percentile on the x-axis. The solid line represents the maximum detection limit of 2,421 MPN/100 mL. $n = 828$.

the maximum detection limits for total coliform and one sample exceeded the maximum detection limit for *E. coli*. Concentrations of nitrate-N, the other targeted contaminant associated with a primary EPA MCL for municipal systems, were generally low compared to previous studies (Bauder *et al.* 1993; Gosselin *et al.* 1997), with only three samples (0.3%) exceeding MCL of 10 mg/L (mean observation = 1.08 mg/L).

Associations between demographic data and bacterial contamination

Since the demographic information (Table 3) as well as the response variable (indicator bacteria presence) were categorical (i.e., non-continuous), the likelihood ratio test was applied to identify associations between income/education on bacteria presence (Figure 3). It was determined that only income was associated with total coliform presence ($p = 0.0145$), although education was almost significantly associated ($p = 0.0537$). Differences in the distribution of total coliform and *E. coli* concentrations by demographic categories were statistically significant for both household income levels and education levels ($p < 0.05$, Kruskal–Wallis test), i.e., the distribution of FIB concentrations observed was statistically different for each income and education category.

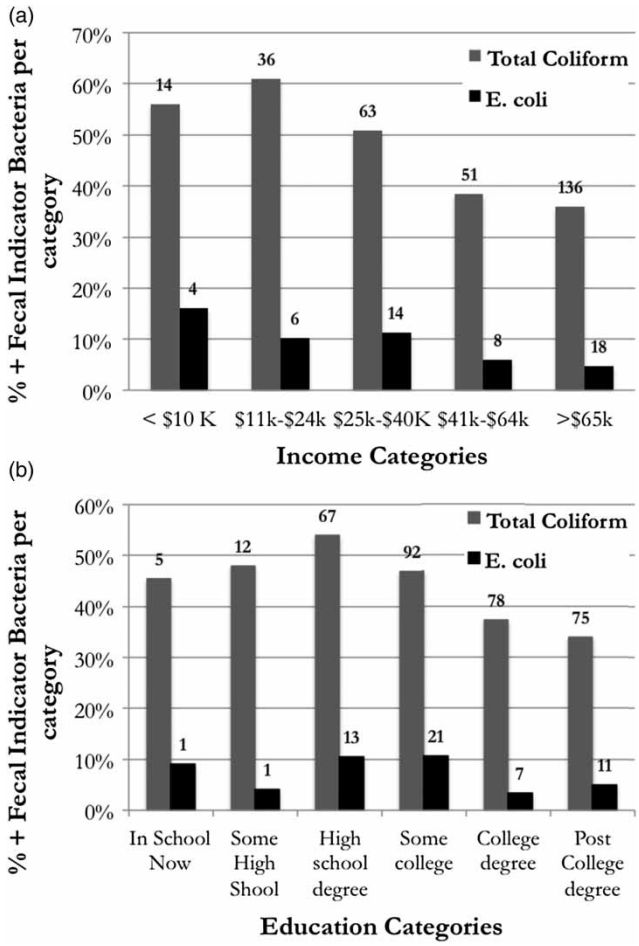

Figure 3 | (a) Percent positive FIB for income categories and (b) percent positive FIB for education categories. *Note:* Households that did not provide income/education information were not included; numbers above each bar denote number of positive sample per category.

Source tracking analyses

Of the 828 samples that were tested for the presence of optical brighteners, 29 tested positive. Characteristics of samples positive for optical brighteners are summarized in Table 4. Although the detection of optical brighteners via fluorometry has been used successfully as a source tracking strategy to detect human sewage contamination in surface waters (Peeler *et al.* 2006), in this study of drinking water supplied by groundwater, only four of the 29 positive samples (14%) also tested positive for *E. coli*. Interestingly, 79% of the optical brightener positive samples came from just two counties in the Northern Neck region of Virginia, Lancaster and

Northumberland, although the total number of samples from these counties ($n = 85$) comprised only 10% of total sample population. None of the optical brightener positive samples from these coastal counties were positive for *E. coli* contamination.

Thirty-five *E. coli*-positive samples were analyzed via qPCR for the human specific HF183 *Bacteroides* target. All 21 samples positive for HF183 were also positive for the general fecal *Bacteroides* marker, GenBac. All samples were also positive for total coliform and *E. coli*, and the observed concentrations in these samples were much higher than the average concentration (Table 4). The majority of HF183-positive samples (57%) were collected from systems dependent on dug/bored wells, which are more prevalent in eastern Virginia, and are generally relatively shallow and more vulnerable to contamination from shallows subsurface flow. Also, the majority of these systems (81%) did not report having a water quality treatment device in place (e.g., sediment filter, chlorinator).

Of the samples described in Table 4, two were positive for both optical brighteners and BacHF183, and so would be considered very likely contaminated by human fecal material (e.g., septage). One of these samples originated from a household that relies on a spring and the other was from a drilled/bored well. Concentrations of total coliform in these samples were >2,421 MPN/100 mL (over detection limit) and 658 MPN/100 mL, and concentrations of *E. coli* were 2,420 MPN/100 mL and 583 MPN/100 mL, respectively. No treatment device was reported in either household and participants indicated that the water had never been tested

Table 4 | Characteristics of samples positive for optical brighteners and/or BacHF183 (human-specific Bacteroides)

		Optical brightener +ve samples ($n = 29$)	BacHF183 +ve samples ($n = 21$)
Avg. TC MPN/100 mL		352	1.715
Avg. *E. coli* MPN/100 mL		111	170
System type	Springs	10%	5%
	Cisterns	0%	0%
	Drilled wells	57%	33%
	Dug/Bored wells	10%	57%
	Unknown well	13%	5%
	No response	7%	0%
Treatment device	Yes	13%	19%
	No	87%	81%
Average well depth ($n = 11$; $n = 14$ reported)		109 m	62 m
Average year built ($n = 12$, $n = 13$ reported)		1984	1972
Water tested	Once	50%	28%
	Never	50%	62%
Objectionable odor		27%	33%
Unnatural color		33%	52%
Water stains application		37%	57%
Visible particles in water		27%	24%
Systems located within 100 feet of:			
	Septic system drain field	17%	22%
	Pit, privy, or outhouse	3%	11%
	Home heating oil storage tank	10%	11%
	Pond or freshwater stream	10%	22%
System is located within 1/2 mile of:	Field crops/Nursery	50%	44%
	Farm animal operation	17%	0%
Drink ($n = 46$; $n = 44$ individuals in home)	Yes	76%	60%
	No	24%	40%

previously. All members of both households indicated that they did drink the water.

DISCUSSION

Almost half of the POU samples submitted by homeowners for this study were positive for total coliforms, and 7% were positive for *E. coli*. These contamination rates are within the range of previously reported study results of private systems in the United States (Allevi *et al.* 2013). Given that the presence of indicator bacteria is considered suggestive of a system breach and/or the presence of fecal material in the water supply, the consistently high rates (>30%) of positive samples across all socio-economic groups (Figures 3(a) and 3(b)) are a cause for concern.

While total coliform results across demographic categories were perhaps indicative of a relationship between socio-economic factors and water quality, these results must be viewed with considerable caution, as the participating population was noticeably skewed toward higher income, more highly educated homeowners (Figure 3; Table 3), and therefore may not be representative of the state or nation as a whole. It is important to recognize that this study represents a very cursory analysis, with identification of the influence of income and/or education relative to other system factors via more advanced statistics limited by a zero-heavy dataset (i.e., majority of samples 'negative') and a population skewed very heavily to the upper income, higher education categories (e.g., only 26 households in the lowest income category, and only 11 in the lowest education category). In this, the results are not dissimilar to or more detailed than Francis (1981) conclusion that 'lower income homes have coliform problems'. However, the identification of a significant difference in observations of concentration between income categories, despite the inherent challenges of the dataset, does support the presence of differences in water quality at the household POU within socio-economic categories. Given that this study was completed over 30 years after the Francis study, it is of interest that these issues may persist in rural communities, given significant gains in municipal water quality and access throughout the nation over the latter half of the twentieth century (Greenberg 2012).

Chemical (optical brighteners) and biological (HF183) markers of human sewage were detected in over 30 samples. While concerning, interpretation of these results is difficult, as previous use of source tracking tools in groundwater studies is quite limited. Although a study of 10 molecular source tracking targets recently identified HF183 as the most effective marker of human sewage available, some cross-reactivity with wildlife has been observed (Layton *et al.* 2013), i.e., the contamination may originate from non-human animals. Conversely, it is important to note that the absence of HF183 does not necessarily ensure the absence of sewage contamination, as associated strains of *Bacteroides*, while common, are not present in all human intestinal tracts due to health and diet (Dethlefsen *et al.* 2008). Whereas municipal sewage discharges represent a large population, rendering the likelihood of *Bacteroides* detection in discharge high, contamination from a septic system represents only a small number of individuals, so the presence of this marker is not guaranteed.

The majority of samples positive for optical brighteners were not positive for *E. coli*, as would be expected, and were detected in samples from the Virginia Coastal Plain physiographic region, which is characterized by sandy underlying soils with high infiltration rates. Differences in underlying geologic and soil composition may have impacted the relative fate and transport (e.g., rates of degradation or adsorption) of fluorescing chemicals versus microorganisms in this region, i.e., fecal bacteria could be moving toward the POU of groundwater wells at a rate slower than optical brighteners. Previous studies have indicated that additional non-wastewater-related anthropogenic chemicals (e.g., diesel fuel, motor oil) do fluoresce (Hartel *et al.* 2008; Hagedorn *et al.* 2011), so contamination by these chemicals would also result in fluorometry-positive samples, although they would have to be present in high concentrations to fluoresce.

CONCLUSION

As in previous studies, the prevalence of FIB-positive samples collected from the POU of homes dependent on private water supplies was high (42% positive for total coliform, 7% positive for *E. coli*), especially given a current EPA maximum contaminant level goal of zero *E. coli* in

samples from municipal systems. Exploration of potential relationships between bacteria-positive samples and participant-reported household income and education level were mixed; although the distribution of observed concentrations differed by income and education levels, the prevalence of positive samples was only statistically significant when comparing total coliform and income level. This might reflect complex interrelationships between socio-economic and environmental factors that will require more targeted examination, particularly among disadvantaged groups or these results could just be a function of the type, age, and condition of a private water supply system that have little or no influence from socio-economic factors.

The present study solely relied on volunteer participation in a Cooperative Extension Program designed to provide educational assistance to families dependent on private water supplies, and the available dataset was skewed toward households with higher education and median income. Nonetheless, the detection of some significant associations despite these inherent limitations recommends further investigation into possible relationships between demographic characteristics and drinking water quality. Confirmation of associations between socio-economic factors and water quality issues will be useful in the design and justification of further extension programs and other public health interventions aimed at improving drinking water quality (e.g., lower cost testing, availability of educational resources, etc.).

Chemical (optical brighteners) and microbial (*Bacteroides* HF183) fecal source tracking targets suggested potential human sewage contamination in 29 and 21 samples, respectively. Although used frequently in surface water studies, the usefulness of these targets in detecting the sources of contamination in private drinking water supplies in order to guide remediation efforts is unclear, particularly with respect to optical brighteners. Four of the 29 samples positive for optical brighteners did not contain detectable *E. coli*, which may reflect relative differences in fate and transport within aquifer media, especially as the majority of these samples were in a single region of Virginia characterized by sandy soils. Further studies are required to resolve this discrepancy, and to determine how source tracking techniques can be best integrated within monitoring strategies to detect drinking water contamination within systems dependent on groundwater sources.

ACKNOWLEDGEMENTS

Funding for this project was provided by the Virginia Tech College of Agriculture and Life Sciences and the United States Department of Agriculture National Institute of Food and Agriculture (Rural Health Safety Education Competitive Grants Program #2011-46100-31115).

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First received 14 November 2013; accepted in revised form 29 April 2014. Available online 29 May 2014