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Association of water quality with soil-transmitted helminthiasis and diarrhea in Nueva Santa Rosa, Guatemala, 2010

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ABSTRACT

Improved water quality reduces diarrhea, but the impact of improved water quality on *Ascaris* and *Trichuris,* soil-transmitted helminths (STH) conveyed by the fecal-oral route, is less well described. To assess water quality associations with diarrhea and STH, we conducted a cross-sectional survey in households of south-eastern Guatemala. Diarrhea was self-reported in the past week and month. STH was diagnosed by stool testing using a fecal parasite concentrator method. We explored associations between *Escherichia coli*-positive source water (water quality) and disease outcomes using survey logistic regression models. Overall, 732 persons lived in 167 households where water was tested. Of these, 79.4% (581/732) had *E. coli*-positive water, 7.9% (58/732) had diarrhea within the week, 14.1% (103/732) had diarrhea within the month, and 6.6% (36/545) tested positive for *Ascaris* or *Trichuris,* including 1% (6/536) who also reported diarrhea. Univariable analysis found a statistically significant association between water quality and STH (odds ratio [OR] = 5.1, 95% confidence interval [CI] = 1.1–24.5) but no association between water quality and diarrhea. Waterborne transmission and effects of water treatment on STH prevalence should be investigated further. If a causal relationship is found, practices such as household water treatment including filtration might be useful adjuncts to sanitation, hygiene, and deworming in STH control programs.

Key words | diarrhea, global health security, Guatemala, helminths, water, water quality

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INTRODUCTION

Diarrhea is a leading cause of morbidity and mortality globally. Diarrhea is of particular concern for children. It causes an estimated 1.3 million deaths among children younger than 5 years annually (Global Burden of Disease Study 2015). In a 2008–2009 national survey, 22.5% of children <5 years of age in Guatemala had diarrhea in the 2 weeks

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preceding the survey (Ministerio de Salud Pública y Asistencia Social 2010). In 2008, diarrheal diseases accounted for approximately 5,000 deaths in Guatemala among all ages, or 6% of the total number of deaths in that country (World Health Organization 2015a).

Although not often a direct cause of mortality, intestinal helminthiasis is one of the most common diseases worldwide, infecting an estimated 1.5 billion (10^9) people and occurring in the poorest and most underserved communities (World Health Organization 2015b). Guatemala has many cases of soil-transmitted helminthiasis (STH), more than most other countries in Latin America (Hotez *et al.* 2008). An estimated 7.9 million people in Guatemala are infected with *Ascaris* and 8.6 million are infected with *Trichuris*, among other intestinal helminths (Hotez *et al.* 2008). STH refers to infection of the intestinal tract by one or more nematodes, including *Ascaris lumbricoides* and *Trichuris trichiura*. Eggs from these worms are passed in the feces and, without adequate sanitation, the eggs contaminate and hatch in the soil. After a development period of a few weeks in the environment, the eggs become infectious and fecal-oral transmission can occur via items contaminated with soil, such as vegetables and dirty hands (Ensink *et al.* 2007; Klapec & Borecka 2012; World Health Organization 2015b). Morbidity from STH is related to the number of worms an individual has and the duration of infection (World Health Organization 2015b). Diagnosis relies on laboratory testing of stool. People with light-intensity infections generally have no overt symptoms (World Health Organization 2015b). However, infections with heavier intensities can cause a range of health effects, particularly in young children, including diarrhea, abdominal pain, anemia, diminished growth, and compromised cognitive development (Albonico *et al.* 2008; World Health Organization 2015b).

Diarrheal diseases can be transmitted by contaminated drinking water, and interventions that improve the microbiologic quality of water are effective diarrhea prevention measures (Clasen *et al.* 2007). However, the association between water quality and STH is not as well characterized. Pooled estimates from a 2014 meta-analysis showed a 33% reduction in the odds of STH infection associated with water, sanitation, and hygiene (WASH) practices or access; most of these studies focused on sanitation but water-related practices and access also appeared to reduce the odds of STH (Strunz *et al.* 2014). While the fecal-oral transmission cycle of *A. lumbricoides* and *T. trichiura* is well known, either through direct ingestion of contaminated soil, such as with young children, or through foodborne transmission from eating uncooked produce contaminated with soil containing infective eggs (Ensink *et al.* 2007; Klapec & Borecka 2012; World Health Organization 2015a, 2015b), much less is known about a possible role for waterborne transmission.

In 2010, we conducted a cross-sectional survey within the *municipio* (county) of Nueva Santa Rosa (NSR) in the *Departamento* (state) of Santa Rosa in Guatemala to estimate the burden of diarrhea and STH in this population. In addition to this primary objective, the cross-sectional survey had several secondary objectives, one of which was a WASH pilot study, which involved household water quality testing. In this paper, we report the findings of the water quality testing from this pilot study and explore the possible associations between fecally contaminated water in NSR and disease, specifically diarrhea and STH.

METHODS

Study area

Guatemala is divided administratively into 8 regions, within which are 22 *Departamentos*. The *Departamento* of Santa Rosa is located south-east of the nation's capital, Guatemala City. The study area, NSR, is one of 14 *municipios* within Santa Rosa. In 2010, Guatemala had an estimated population of 14.4 million and NSR had an estimated population of 31,044 people (Instituto Nacional de Estadística 2010). The last national census in 2002 counted 5,918 households with an average of 4.8 persons per household in NSR (Instituto Nacional de Estadística 2002).

Sampling method and study design

We conducted a cross-sectional survey in NSR from August through September 2010. Potential residential-associated (PRA) roofs were identified on high-resolution aerial photographs from the Instituto Geográfico Nacional (National

Geographic Institute) of Guatemala. These photos covered the entire area of NSR. The aerial photos were taken in 2006 and were overlaid with the 2002 census tract maps for NSR to identify the boundaries of the *municipio*. Next, a grid of 200 m × 200 m cells and a topographic map with global positioning system coordinates were overlaid. PRA roofs were then identified in the photos according to *a priori* criteria, namely shapes recognized as man-made constructions ranging in size from 16–150 m^2, which was believed to be the size range appropriate for buildings that could represent houses, kitchens, sleeping rooms, living rooms, or dining rooms. Such roofs were then geo-located. In total, 10,770 PRA roofs were identified and formed the sampling frame.

The main cross-sectional survey, to be described in a subsequent paper, required a sample of 387 PRA roofs that were randomly selected from among the 10,770 PRA roofs in the sampling frame. Study staff then attempted to find these 387 roofs in the field and identify their associated households. Subsequently, informed consent for household participation was obtained from the head of each household. A household spokesperson (preferably the female head of household) was interviewed on behalf of all household occupants. However, anyone was free to speak on their own behalf about his/her own health and behaviors. The household spokesperson was surveyed using a standardized questionnaire.

Diarrhea was self- or proxy-reported. Four questions based on ones used for local hospital-based public health surveillance activities were asked. First, for each household member, the spokesperson or individual was asked (1) if he/she had diarrhea (loose stools) in the past month. If an affirmative answer was given to this screening question, three additional questions were asked: (2) how many days ago was the last episode of diarrhea, (3) how many days in total did the diarrhea last, and (4) during the worst day of diarrhea, how many loose stools did the person have. Diarrhea was defined as ≥3 loose stools in a 24-hour period. Those individuals with ≥3 loose stools on their worst day of diarrhea may have experienced this worst day at any time in the past month if he/she had prolonged diarrhea or if he/she had multiple episodes of diarrhea in the past month. Consequently, even if the individual reported diarrhea/loose stools in the past 7 days, the worst day of diarrhea with ≥3 loose stools in 24 hours may have occurred at an earlier time. Therefore, we developed two case definitions for diarrhea: (1) diarrhea (loose stools) in the past 7 days; and (2) ≥3 loose stools within a 24-hour period in the past month.

For STH, a single stool specimen from a participant who was ≥1 year old was collected, preserved in the field in formalin, and processed at the Universidad del Valle de Guatemala (UVG) laboratory in Guatemala City using the Mini Parasep® Fecal Parasite Concentrator (FPC) method (Apacor 2016). This specimen was then microscopically examined for hookworm, *A. lumbricoides* and *T. trichiura*. We removed hookworm positive participants from the drinking water analysis since the primary mode of hookworm transmission is not fecal oral; rather, hookworm infections occur through the skin. Henceforth, *A. lumbricoides* and *T. trichiura* are referred to collectively as STH (World Health Organization 2006). Hookworm infections were excluded, but *A. lumbricoides* and *T. trichiura* were included.

Household water quality was measured by quantity of *Escherichia coli*, a marker of fecal contamination, in a water sample taken directly from the household's main source of drinking water (i.e. a faucet connected to a municipal water system, a backyard well, a river, a lake, commercially bottled water, or another source as indicated) – this sample was not collected from a storage container in the house. The only exception was when the source water was commercially bottled water. The water sample was then taken from the commercially bottled water that was stored at the home. One source water sample per household was taken for analysis. The water sample (approximately 100 mL) was poured into a previously unopened water collection container (Hach® bottle), according to accepted practices (World Health Organization 1997). The Hach® bottles did not contain thiosulfate that would have neutralized any chlorine present in the water (Hach Company 2016) thus allowing for free chlorine residual testing in the field using a Hach® Chlorine (Free) Test Kit (Hach Company 1997). The water samples were then stored in insulated coolers at 4 °C, which were transported to the UVG laboratory at the end of each day where they were tested for *E. coli* by the Colilert® Most Probable Number (MPN) method utilizing the Quanti-Tray 2000® Enumeration Procedure (IDEXX, Westbrook, Maine, USA).

Statistical analysis

The exposure variable was a dichotomous measure of water quality as indicated by either a positive test for *E. coli* (MPNs > 0) or a negative test (MPNs = 0). The outcomes of interest were also dichotomous measures: (i) any occurrence of diarrhea (loose stools) that were self- or proxy-reported in the past 7 days, (ii) self-reported ≥3 loose stools within a 24-hour period in the past month, and (iii) laboratory-confirmed STH (*A. lumbricoides* and/or *T. trichiura*). Variables were dichotomized because of limited sample size, power, and non-normal distributions.

Several possible confounders were considered, including age, population density, household crowding, type of material covering the floor, water source, water treatment, and socioeconomic status (SES), which was measured by household possessions, number of animals, and maternal education. Age was categorized as young children (<5 years old), school-aged children (5–14 years old), and adults (persons ≥15 years old). However, when dichotomized, age was divided into children (<15 years old) versus adults (≥15 years old). Lower population density areas were defined as those where <1,000 people per km^2 lived (Hay *et al.* 2005). Household crowding was defined as >2.5 people per bedroom, which was the median value in the dataset. We defined finished floors as those covered with wood, vinyl, ceramic tiles, cement, carpet, or brick. Improved water sources included taps, boreholes, protected wells, rainwater, and commercially bottled water as defined by the WHO Joint Monitoring Program for Water and Sanitation (World Health Organization 2008). Household water treatment was a self-reported variable. Water samples from sources that could possibly be chlorinated were also tested for free chlorine. The WHO recommends a minimum free chlorine residual level of 0.2–0.5 mg/L at the point of delivery for water that is centrally treated and 0.2 mg/L in stored household water treated by chlorination (World Health Organization 1997). We grouped *E. coli* measurements either by WHO classification of water contamination for sanitary inspection risk score (<1, 1–9, 10–99, ≥100 MPN/100 ml) for the descriptive analysis (2011) and as a dichotomous variable when examining possible associations (<1 vs. ≥1 *E. coli* colony forming units [CFU]). Several variables were identified as being potentially related to SES, including maternal education category, household income, building characteristics of the home, household ownership of items such as electronic and transportation devices, and variables indicating whether or not specific animals were owned or slept indoors. A factor analysis (Hendrickson & White 1964) using a promax rotation (a procedure which simplifies the factor structure and hence makes its interpretation easier and more reliable) was performed on these SES variables to create new variables, or 'factors,' that captured the variability in household SES by combining individual SES variables. Of the 33 SES variables considered, five variables were excluded due to a small number of households reporting characteristics such as ownership of a particular item or animal. Another 10 variables were excluded because they did not contribute strongly to the factor scores (loading <0.4). The remaining 18 variables were summarized by two representative SES factors. Using the minimum eigenvalue criterion for choosing the number of factors, three factors would have been chosen, but two of these factors would have been related to animal ownership and been strongly influenced by only three variables each. It was determined that two factors adequately captured SES variability, one factor representing household possessions ('household possessions factor') and the other primarily representing household animal possession ('animal ownership factor'). Scores for both factors were dichotomized at zero, where natural breaks in the distributions occurred, with higher scores indicating higher household economic status or greater animal/livestock ownership.

We used a Rao-Scott chi square test, accounting for household clustering, and weighted by the number of roofs per household (i.e., the number of roofs in the sampling frame associated with each particular household that was surveyed) to compare the demographics and household environmental characteristics of the individuals included in the water quality and disease analyses group with those of the individuals surveyed but not included in these analyses because they either did not have household water testing or stool STH testing results. The associations between water quality and each disease outcome were separately explored with univariable modeling using logistic regression, accounting for household clustering and weighting by the number of roofs per household. Multiple logistic regression models were used to adjust for possible confounders. Each model included a single confounder because of small numbers (Budtz-Jorgensen *et al.* 2007). The confounders were defined as those variables causing a greater than 10% change in the adjusted odds ratio (aOR) compared to the odds ratio from the univariable model regardless of their statistical significance. All statistical tests were performed at significance level of 0.05. All analyses were performed using SAS v9.3 (SAS Institute, Carey, NC).

Human subject protections

This study was conducted in accordance with the ethical standards of the Helsinki Declaration of the World Medical Association. The protocol was approved by the Ethics Committee of the UVG in Guatemala City, Guatemala (protocol 038-04-2010, approval date July 19, 2010) and the Institutional Review Board of the Centers for Disease Control and Prevention (CDC) in Atlanta GA, USA (protocol 5936, approval date June 18, 2010). Written or thumbprint records of free and informed consent for household participation and for individual participation were obtained from participants or their legal representatives before enrolment into the study and collection of stool specimens and water samples.

RESULTS

Population description

Of the 387 randomly selected PRA roofs, 12 (3.1%) were not found and 65 (16.8%) were not associated with residential structures. Of the remaining 310 roofs, four roofs were associated with multiple households (nine households in all), for a total of 315 households, of which 255 (81.0%) were occupied with an adult at the time of the survey. Overall, 44 occupied households (17.3%) declined to participate in the survey. Among the remaining 211 consenting households, seven (3.3%) were excluded from analysis because of data incompleteness or inconsistent answers that could not be resolved. Therefore, from 387 PRA roofs, 204 households, representing 920 individuals, were included in the final cross-sectional survey dataset.

Water quality results

Of the 204 households, 167 (81.9%) consented and provided source drinking water samples for *E. coli* testing. The main household drinking water sources identified by the 167 households at the time of testing included bottled water (53, 32%), piped water into the dwelling (49, 29%), and public taps (22, 13%) (Table 1). Of the 167, 159 (95.2%) households also had free chlorine residual testing. Source water samples from a wide variety of different water sources tested positive for *E. coli* in 75.4% ($n = 126$) of households (Table 1). Among the 126 *E. coli* positive household source water samples, 37.3% ($n = 47$) had 1–9 MPN/100 mL, 27.0% ($n = 34$) had 10–99 MPN/100 mL, and 35.7% ($n = 45$) had $\geq$100 MPN/100 mL (Figure 1). Free chlorine residual concentrations were $\geq$0.2 mg/L (range, 0.20–0.60 mg/L) in only 3.1% ($n = 5$) of water samples (four from in-home taps and one from commercially bottled water), with a median of 0.50 mg/L.

Water quality testing was not performed in 37 households with 188 residents. These individuals were excluded from diarrhea- and STH-related analyses. The remaining data from 732 individuals was analyzed for associations between water quality and diarrhea (the diarrhea analysis sub-group). In this sub-group, 581 (79.4%) of participants had *E. coli*-positive water indicating fecal contamination of their water (Table 2). Of these, 58 (7.9%) reported diarrhea in the past week, and 103 (14.1%) reported $\geq$3 loose stools within a 24-hour period in the past month. Of the 732 people with water quality testing, 187 were excluded from the STH analysis sub-group (Figure 2): 176 (24.0%) who had no stool testing, two (0.3%) who tested positive for hookworm, and nine (1.2%) who were <1 year old. In the STH analysis sub-group, data from the remaining 545 participants were analyzed: 79.3% (432) had *E. coli*-positive water and 6.6% (36) tested positive for *A. lumbricoides* and/or *T. trichiura*.

Prior to doing the diarrhea analysis, we checked whether there were differences among the group of individuals without water testing ($n = 188$) 'excluded' as compared to those with water testing ($n = 732$) 'included.' We found only three significant differences between the 'excluded' and 'included' groups (Figure 2) – a significantly higher percentage of the 'excluded' individuals used public taps (62.8% vs. 10.0%, $p < 0.001$) and stored their water at home (98.4% vs. 82.0%, $p = 0.02$). However, a significantly lower

Table 1 | Types and quality[a] of main household drinking water sources – Nueva Santa Rosa, Guatemala, 2010

Main source of drinking water at the time of water quality testing	Number of households using this drinking water source, *N* (%)	Number (percentage) of households with source water testing positive for *E. coli*[a], *n* (%)
Piped water into dwelling (private tap)	49 (29.3)	42 (85.7)
Piped water to yard/plot (private tap)	8 (4.8)	8 (100.0)
Public tap/standpipe (public tap)	22 (13.2)	22 (100.0)
Tubewell/borehole – private	4 (2.4)	3 (75.0)
Tubewell/borehole – public	2 (1.2)	2 (100.0)
Protected dug well – private	1 (0.6)	1 (100.0)
Protected dug well – public	1 (0.6)	1 (100.0)
Unprotected dug well – public	2 (1.2)	2 (100.0)
Protected spring – private	3 (1.8)	3 (100.0)
Protected spring – public	3 (1.8)	3 (100.0)
Unprotected spring – private	3 (1.8)	3 (100.0)
Unprotected spring – public	9 (5.4)	9 (100.0)
Rainwater collection – private	1 (0.6)	1 (100.0)
Rainwater collection – public	3 (1.8)	2 (66.7)
Bottled water or sachet water	53 (31.7)	21 (39.6)
Surface water (river, dam, lake, pond, stream, canal, irrigation channels)	2 (1.2)	2 (100.0)
Other[b]	1 (0.6)	1 (100.0)
Total	**167**	**126 (75.4)**

[a]Water quality was measured by the presence of *Escherichia coli* (*E. coli*), a marker of fecal contamination, in a water sample taken from the household's main source of drinking water (i.e. a faucet connected to a municipal water system, a backyard well, a river, a lake, commercially bottled water, or another source). This sample (approximately 100 mL) was then poured into a previously unopened water collection container (Hach® bottle), according to accepted practices (World Health Organization 1997).

[b]The spokesperson for one household said his main source of drinking water was the water his neighbor gave to him. This water, collected on the participating household's property, tested positive for *E. coli*.

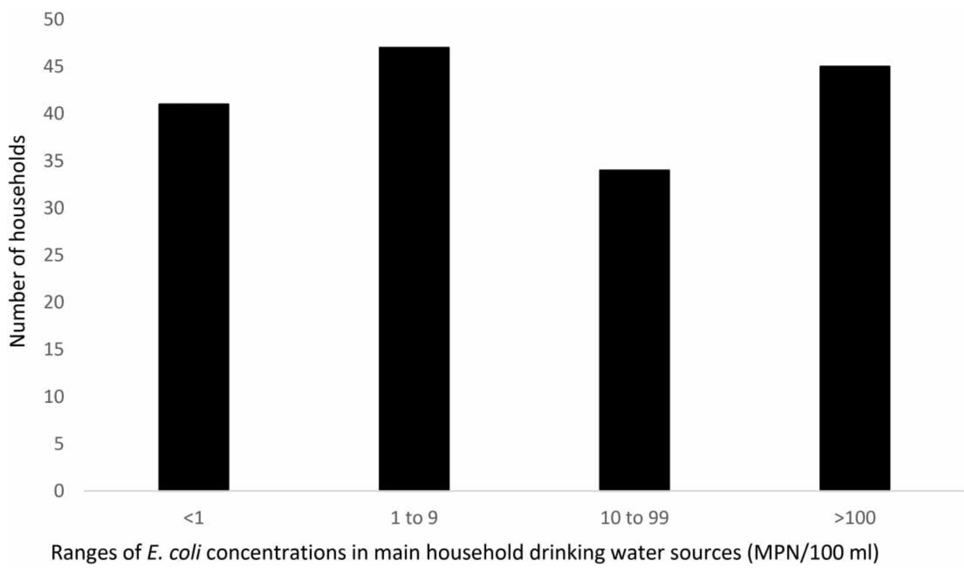

Figure 1 | Distribution of main household drinking water *Escherichia coli* concentrations – Nueva Santa Rosa, Guatemala, 2010.

Table 2 | Demographic and household environmental characteristics of participants with poor water quality (as measured by positive *Escherichia coli*) among different study sub-groups – Nueva Santa Rosa, Guatemala, 2010

Variable	Overall study Total	Water quality testing (diarrhea analyses) ($n = 732$) Total	Positive *E. coli*	Water quality and stool testing (STH[a] analyses) ($n = 545$) Total	Positive *E. coli*
Household possessions factor >0[b]	331/852 (38.9%)	228/682 (33.4%)	192/682 (28.2%)	212/504 (42.1%)	140/504 (27.8%)
Animal ownership factor >0[c]	164/852 (19.3%)	117/682 (17.2%)	100/682 (14.7%)	88/504 (17.5%)	78/504 (15.5%)
Live in lower population density area[d]	141/920 (15.3%)	109/732 (14.9%)	97/732 (13.3%)	74/545 (13.6%)	66/545 (12.1%)
Crowded home[e]	459/920 (49.9%)	349/732 (47.7%)	307/732 (41.9%)	256/545 (47.0%)	227/545 (41.7%)
Finished floors[f]	573/916 (62.6%)	472/728 (64.8%)	336/728 (46.2%)	340/541 (62.9%)	236/541 (43.6%)
Improved water source[g]	789/909 (86.8%)	612/721 (84.9%)	463/721 (64.2%)	454/537 (84.5%)	343/537 (63.9%)
Store water at home	782/916 (85.4%)	597/728 (82.0%)	487/728 (66.9%)	457/541 (84.5%)	373/541 (68.9%)
Treat water[h]	287/920 (31.2%)	235/732 (32.1%)	186/732 (25.4%)	173/545 (31.7%)	133/545 (24.4%)
E. coli-positive source water	581/732 (79.4%)	581/732 (79.4%)	NA	432/545 (79.3%)	NA
Chlorine residual-positive water[i]	19/714 (2.7%)	19/705 (2.7%)	4/705 (0.6%)	15/524 (2.9%)	4/524 (0.8%)
Age					
0–4 years old	97/913 (10.6%)	79/726 (10.9%)	67/726 (9.2%)	63/545 (11.6%)	56/545 (10.3%)
5–14 years old	250/913 (27.4%)	193/726 (26.6%)	160/726 (22.0%)	151/545 (27.7%)	124/545 (22.8%)
≥ 15 years old	566/913 (62.0%)	454/726 (62.5%)	350/726 (48.2%)	331/545 (60.7%)	252/545 (46.2%)

[a]Soil-transmitted helminthiasis (STH) diagnosed in a single stool specimen, which was processed using the Mini Parasep® FPC method, examined microscopically, and found to be positive for *Ascaris lumbricoides* and/or *Trichuris trichiura*.
[b]The household possessions factor represented household possessions, mother's education, and household wall construction.
[c]The animal ownership factor represented a household's livestock ownership.
[d]Lower population density defined as population density <1,000 people/km^2.
[e]Crowded homes defined as >2.5 people per bedroom, the median of people per bedroom in the dataset.
[f]Finished floors include wood, vinyl, ceramic tiles, cement, carpet, and brick versus earthen (sand, dung, straw, or saw dust).
[g]Improved water sources included taps, boreholes, protected wells, rainwater, and commercially bottled water.
[h]Water treatment was self-reported as anything done to the water after collection to make drinking water safer for consumption, including boiling, adding bleach/chlorine, adding iodine, straining water through a cloth, using a water filter (ceramic, sand, composite), using solar disinfection, letting water stand/settle, or adding a flocculant.
[i]The World Health Organization recommends a minimum free chlorine residual level of 0.2–0.5 mg/L at the point of delivery for water that is centrally treated and 0.2 mg/L in stored household water treated by chlorination (World Health Organization 2011).

percentage of 'excluded' individuals used commercially bottled water (3.2% vs. 27.7%, $p = 0.01$). There was no difference between the 'excluded' vs. 'included' groups for the diarrhea analyses when all improved water sources (as previously defined) were combined, (94.1% among excluded vs. 83.6%, $p = 0.33$). In the STH analysis, we made the same comparison between 'excluded' individuals without water and stool testing ($n = 375$) and 'included' individuals with both water and stool testing ($n = 545$); no statistically significant differences were found between the two groups.

Disease outcomes: diarrhea and STH

Comparing all three outcomes (diarrhea in the past 7 days, diarrhea in the past month, and STH) among participants with both water and stool testing, 9.7% (52/537) reported diarrhea in the 7 days before the survey, 17.0% (91/536) reported ≥3 loose stools in a 24-hour period in the month before the survey, and 6.6% (36/545) tested positive by FPC on a single stool specimen for *A. lumbricoides* and *T. trichiura*. Among the 536 individuals with stool testing results and complete information, six had diarrhea (by either or both definitions) and STH (Figure 3).

The majority of participants evaluated in the diarrhea analyses and the STH analyses were ≥15 years old and had little means, as measured by the household possessions factor and the animal ownership factor (Table 2). Most people had access to an improved water source (86.8%) (Table 2), including 31.7% of households that had commercially bottled water as their primary water source (Table 1).

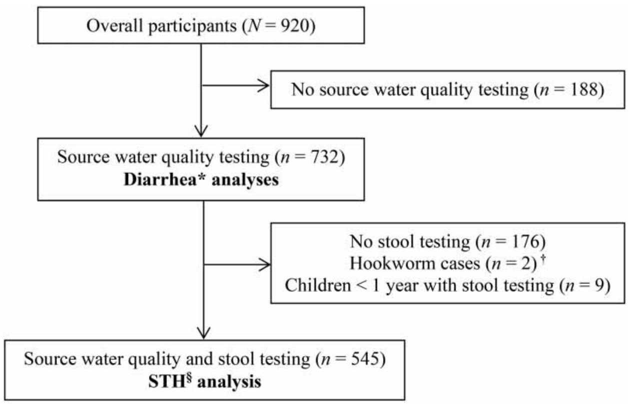

Figure 2 | Sub-groups used for analyses of associations between source water quality and disease outcomes – Nueva Santa Rosa, Guatemala, 2010. *Self- or proxy-reported diarrhea. Two definitions used for analyses: (1) diarrhea (loose stools) in the past 7 days, and (2) ≥3 loose stools within a 24-hour period in the past month. †Hookworm was excluded because it is not transmitted by the fecal-oral route. Children <1 year of age who had stool testing but for a bacterial and viral sub-study were excluded. §Soil-transmitted helminthiasis (STH) diagnosed in a single stool specimen, which was processed using the Mini Parasep® FPC method, examined microscopically, and found to be positive for *Ascaris lumbricoides* and/or *Trichuris trichiura*. These two parasites are transmitted by the fecal-oral route.

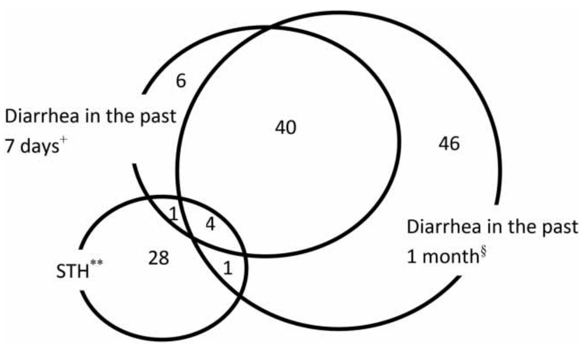

Figure 3 | Overlap in participants with cases of diarrhea in the past 7 days and 1 month and cases of soil-transmitted helminths (STH) who also had source water quality and stool testing*, Nueva Santa Rosa, Guatemala, 2010. *Since not every participant had source water quality, stool testing, and diarrhea reported, not all participants are represented in this figure. +Self- or proxy-reported diarrhea (loose stools) in the 7 days before the survey. §Self- or proxy-reported ≥3 loose stools within a 24-hour period on at least one day in the past month before the survey. **Soil-transmitted helminthiasis (STH) diagnosed in a single stool specimen, which was processed using the Mini Parasep® FPC method, examined microscopically, and found to be positive for *Ascaris lumbricoides* and/or *Trichuris trichiura*.

The majority (79.4%) of source water samples tested had fecal contamination as indicated by the presence of *E. coli* and, as noted previously, very few people had recommended levels of free chlorine residuals (≥0.2 mg/L) in their source water (2.7%).

Demographic and household environmental characteristics varied among those with diarrhea vs. those with STH (Table 3). For example, the percentage of individuals in the upper category of the household possessions factor (i.e., household possessions factor >0), was larger among those who reported diarrhea (40.7% and 44.8% in the past 7 days and past month, respectively) than among those with STH (8.6%). On the other hand, the percentage of individuals in the upper category of the animal ownership factor, was lower in individuals with diarrhea (9.3% and 11.5% in the past 7 days and past month, respectively) than in individuals with STH (34.3%). Differences were also noted for population density, household crowding, and age, but were greatest for finished floors: 66.7% and 68.3% of those with diarrhea had finished floors versus only 22.2% of those with STH.

In univariable modelling, no statistically significant association was found between fecal contamination of water and either diarrhea in the past 7 days or in the past month (Table 4). *E. coli* contamination of water was associated with increased odds of STH infection, based on the univariable modelling (odds ratio [OR] = 5.14, confidence interval [CI] = 1.08–24.47, $p = 0.04$) (Table 4). This modeling showed that the household possessions factor, finished floors, crowded homes, improved water sources, animal ownership factor, and household water storage were confounders that had an effect on the association between water quality and STH (Table 5). Conversely, being an adult (≥15 years old), reporting drinking treated water, living in a lower population density area, and having chlorine-positive water did not confound the associations between water quality and STH.

Because of sparse data and issues with quasi separation, we could not check for interaction between confounders or between confounders and water quality. Our analyses using survey methods did not account for the sparse data issue that occurred with STH as the outcome in this analysis (only one individual had STH infection and *E. coli* negative water). Therefore, we repeated the analyses using exact logistic regression, addressing the sparse data issues (but not adjusting for the sample design, i.e., clustering and weighting). The results showed the same general trend and the same statistically significant factors in our previous analyses remained significant when accounting for the sparse data issues alone (data not shown).

Table 3 | Demographic and household environmental characteristics by disease outcomes – Nueva Santa Rosa, Guatemala, 2010

	Diarrhea in past 7 days		≥3 loose stools in 24 hours in past month		STH[a]-positive stool	
Variable	***n/N***	**(%)**	***n/N***	**(%)**	***n/N***	**(%)**
Household possessions factor >0[b]	22/54	(40.7%)	43/96	(44.8%)	3/35	(8.6%)
Animal ownership factor >0[c]	5/54	(9.3%)	11/96	(11.5%)	12/35	(34.3%)
Live in lower population density area[d]	9/58	(15.5%)	17/103	(16.5%)	2/36	(5.6%)
Crowded home[e]	27/58	(46.6%)	50/103	(48.5%)	28/36	(77.8%)
Finished floors[f]	38/57	(66.7%)	69/101	(68.3%)	8/36	(22.2%)
Improved water source[g]	45/57	(78.9%)	78/101	(77.2%)	23/36	(63.9%)
Store water at home	49/57	(86.0%)	91/101	(90.1%)	30/36	(83.3%)
Treat water[h]	17/58	(29.3%)	29/103	(28.2%)	8/36	(22.2%)
E. coli-positive source water	47/58	(81.0%)	87/103	(84.5%)	33/36	(91.7%)
Chlorine residual-positive water[i]	3/56	(5.4%)	4/98	(4.1%)	1/34	(2.9%)
Age						
0–4 years old	13/58	(22.4%)	23/103	(22.3%)	4/36	(11.1%)
5–14 years old	16/58	(27.6%)	25/103	(24.3%)	18/36	(50.0%)
≥ 15 years old	29/58	(50.0%)	55/103	(53.4%)	14/36	(38.9%)

[a]Soil-transmitted helminthiasis (STH) diagnosed in a single stool specimen, which was processed using the Mini Parasep® FPC method, examined microscopically, and found to be positive for *Ascaris lumbricoides* and/or *Trichuris trichiura*.
[b]The household possessions factor represented household possessions, mother's education, and household wall construction.
[c]The animal ownership factor represented a household's livestock ownership.
[d]Lower population density defined as population density <1,000 people/km^2.
[e]Crowded homes defined as >2.5 people per bedroom, the median of people per bedroom in the dataset.
[f]Finished floors include wood, vinyl, ceramic tiles, cement, carpet, and brick versus earthen (sand, dung, straw, or saw dust).
[g]Improved water sources included taps, boreholes, protected wells, rainwater, and commercially bottled water.
[h]Water treatment was self-reported as anything done to the water after collection to make drinking water safer for consumption, including boiling, adding bleach/chlorine, adding iodine, straining water through a cloth, using a water filter (ceramic, sand, composite), using solar disinfection, letting water stand/settle, or adding a flocculant.
[i]The World Health Organization recommends a minimum free chlorine residual level of 0.2–0.5 mg/L at the point of delivery for water that is centrally treated and 0.2 mg/L in stored household water treated by chlorination (World Health Organization 2011).

Table 4 | Associations between poor source water quality (as measured by positive *Escherichia coli*) and disease outcomes – Nueva Santa Rosa, Guatemala, 2010

Disease outcome	Odds ratio (OR)	95% confidence interval	*P* value
Diarrhea (loose stools) in the past 7 days[a]	0.92	[0.43, 1.98]	0.83
≥3 loose stools in 24 hours in the past month[b]	1.24	[0.60, 2.57]	0.56
STH[c]	**5.14**	**[1.08, 24.47]**	**0.04**

[a]Self- or proxy-reported symptoms in the 7 days before the survey.
[b]Self- or proxy-reported symptoms on at least one day in the month before the survey.
[c]Soil-transmitted helminthiasis (STH) diagnosed in a single stool specimen, which was processed using the Mini Parasep® FPC method, examined microscopically, and found to be positive for *Ascaris lumbricoides* and/or *Trichuris trichiura.* These two parasites are transmitted by the fecal-oral route.

DISCUSSION

Our findings of an association between water quality, using the Colilert® MPN method to evaluate *E. coli* concentration, and STH are intriguing. Few studies have shown indirect associations between water quality and STH infection, i.e., that odds of STH infection are reduced among those using treated water (Aimpun & Hshieh 2004; Ahmed *et al.* 2011; Wang *et al.* 2012; Strunz *et al.* 2014). A more direct measurement of water contamination when examining its relationship to STH is detection of helminth eggs in the water. Two studies have measured STH in drinking water sources. Eggs of *A. lumbricoides* and *T. trichiura* were

Table 5 | Associations between poor source water quality (as measured by positive *Escherichia coli*) and soil-transmitted helminthiasis, adjusting for possible confounders – Nueva Santa Rosa, Guatemala, 2010

Disease outcome	Univariable analysis (unadjusted)	Odds ratio (OR)	95% confidence interval (CI)	*P* value
STH[a]	–	5.14	[1.08, 24.47]	0.04
Disease outcome	Multivariable logistic regression analysis (adjusted by one variable at a time)	Adjusted odds ratio (aOR)	95% CI	*P* value
STH	Household possessions factor >0[b]	2.07	[0.48, 8.92]	0.33
	Finished floors[c]	2.21	[0.44, 11.06]	0.33
	Crowed home[d]	3.55	[0.68, 18.39]	0.13
	Improved water source[e]	3.76	[0.74, 19.18]	0.11
	Animal ownership factor >0[f]	4.10	[0.83, 20.33]	0.08
Within 10% of the unadjusted OR	≥15 years old	4.67	[0.99, 22.10]	0.05
	Treat water[g]	5.21	[1.08, 25.02]	0.04
	Live in lower population density area[h]	5.48	[1.15, 26.21]	0.03
	Chlorine residual-positive water	5.53	[1.01, 30.20]	0.04
	Store water at home	5.72	1.22, 26.76	0.03

[a]Soil-transmitted helminthiasis (STH) diagnosed in a single stool specimen, which was processed using the Mini Parasep® FPC method, examined microscopically, and found to be positive for *Ascaris lumbricoides* and/or *Trichuris trichiura.* These two parasites are transmitted by the fecal-oral route.
[b]The household possessions factor represented household possessions, mother's education, and household wall construction.
[c]Finished floors include wood, vinyl, ceramic tiles, cement, carpet, and brick verses earthen (sand, dung, straw, or saw dust).
[d]Crowded homes defined as >2.5 people per bedroom, the median of people per bedroom in the dataset.
[e]Improved water sources included taps, boreholes, protected wells, rainwater, and commercially bottled water.
[f]The animal ownership factor represented a household's livestock ownership.
[g]Water treatment was self-reported as anything done to the water after collection to make drinking water safer for consumption, including boiling, adding bleach/chlorine, adding iodine, straining water through a cloth, using a water filter (ceramic, sand, composite), using solar disinfection, letting water stand/settle, or adding a flocculant.
[h]Lower population density defined as population density <1,000 people/km^2.

recovered from water storage tanks in Hyderabad, India (Jonnalagadda & Bhat 1995) and from household earthenware water storage containers in Egypt (Khairy *et al.* 1982). While neither of these studies demonstrated causal effect, it is biologically possible that helminth eggs could be transmitted via water. Water chlorination, a common method of water treatment, might not be effective against helminth eggs because of their chlorine resistance (Bandala *et al.* 2012) and additional or alternative household water treatment methods such as filtration may need to be considered. While likely not a main mode of transmission, waterborne transmission of STH might be a possible mechanism of infection warranting further investigation as a component of the WASH intervention package that could be deployed to augment current STH control efforts to help prevent STH infection and re-infection. In addition to effective water treatment interventions, the water component of such a WASH intervention package should also include material supplies and education to enable safe water storage and prevent stored water contamination in the household. Further, WASH partnerships should help communities secure access to adequate quantities of water for daily needs beyond drinking, (e.g., hygiene, food preparation and cooking, cleaning, laundry, etc.).

In this evaluation, we observed a significant association between the presence of *E. coli* in source drinking water and STH infection. No statistically significant associations were detected between the presence of *E. coli* in drinking water and diarrhea, the symptom most commonly associated with fecal contamination of water. This study has several limitations. First, diarrhea was a proxy- or self-reported measure influenced by family spokesperson or individual recall, understanding of the survey questions, or personal knowledge if reporting about another person. In particular, the recall period, which was the past 7 days or the past month, for diarrheal episodes in children likely gives an underrepresentation, especially of milder disease (Zafar *et al.* 2010). Second, this cross-sectional study was not designed to capture practices over time. Households whose members were recently sick with diarrhea may have been prompted to treat their water, reversing the relationship between water quality and diarrhea in the household. We tried to investigate this further with the data we had, but were unable to detect differences in reported recent household water treatment practices among those with diarrhea as compared to those without. Third, we were unable to subset our analyses for persons with diarrhea onset more recently than 7 days and used the simple presence or absence of detectable *E. coli* in a 100 mL sample of water as an indicator of water quality because of limitations of sample size and power – this was a sub-study within a WASH pilot study that itself was embedded within a larger cross-sectional survey. Previous studies have shown that a stronger correlation between water quality and diarrhea is found when samples are tested close to the time of the diarrheal episode and when higher cutoffs for *E. coli* contamination, such as >100 or >1,000 *E. coli* per 100 mL are used to define water quality (Luby *et al.* 2015). Lastly, there was limited power to detect a statistically significant association between water quality and diarrhea since this was a sub-study within a WASH pilot study that itself was embedded within a larger cross-sectional survey.

However, the study power for the STH/water-quality analysis was also limited. In this sub-study, logistical constraints limited us to testing a single stool specimen, although a single stool test is known to be less sensitive for detecting STH infections than testing two or three stools from the same person (Knopp *et al.* 2008). Therefore, the STH results in this study likely under-represent the true burden of disease, biasing the results towards the null. Furthermore, this study relied on the FPC method of diagnosis, which is less sensitive than the Kato Katz method (Goodman *et al.* 2007). Thus, the observed association in this pilot study between water quality and STH but not between water quality and diarrhea is even more surprising. However, unlike diarrhea, STH infection was evaluated by an objective laboratory test and was therefore not subject to recall, courtesy, or other biases that can influence self-reported outcomes or behaviors. Additionally, the association between water quality and STH infection was found even with a smaller sample size for the STH/water-quality analysis than that for the diarrhea/water-quality analysis because stool testing was performed on even fewer people.

There are many potential risk factors for STH, including being school-aged, living in poverty, lacking access to sanitation, and drinking untreated water (Strunz *et al.* 2014). We observed that lower SES, unfinished floors in the home, crowding in the home, and using unimproved sources for drinking water appeared to be confounders in our models, modifying the associations between water quality and STH (Table 5). These confounders might truly have stronger effects on STH infection than water quality. Alternatively, there could be a more complicated web of associations among the entangled factors of poverty, poor housing, poor water quality, and disease. SES is a marker for a complex group of poorly understood circumstances that seems to affect the risk for many diseases. Unfortunately, the small sample and sparse data in this pilot study limited our ability to check for effect modification and further explore the possible association between water quality and STH.

CONCLUSIONS

The association between water quality and STH infection observed in this pilot study warrants further study. Replication of these results is important since the association between water quality and STH was found to be modulated by finished floors, crowded homes, obtaining water from an improved water source, storing water at home, and by factors representing household possessions and animal ownership. A larger sample size would allow for more exploration of confounders. Additionally, data that are more robust could be gathered using a more sensitive STH testing procedure such as Kato Katz and by collecting more stool samples per individual, although both of these present significant logistical challenges in the field. Additionally, the possibility of STH contamination of drinking water needs further investigation, including determining the frequency and degree of such contamination, the correlation of STH contamination with markers of fecal contamination, the viability of STH eggs recovered from water samples, and the infectious doses of *A. lumbricoides* and *T. trichiura* eggs in drinking water.

If a causal relationship between water quality and STH infection is found, water treatment might be a useful adjunct to sanitation, hygiene, and deworming in STH control programs. Mass drug administration programs are the cornerstone of STH control programs, but unless underlying environmental factors that greatly influence the likelihood of transmission, such as sanitation and hygiene, are addressed, reinfection is likely to occur (Freeman *et al.* 2013). Ensuring microbial water quality is important in preventing many diseases; STH might also belong in this group and therefore water treatment might be one of multiple WASH-related tools to add to the STH control program armamentarium. Given the high human health burden caused by waterborne diseases and STH, efforts to prevent and control these diseases will advance global health. We believe further investigation is warranted into the association between water quality and STH observed here. If a causal relationship is found, advancing water treatment interventions that could contribute STH control would be vital.

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DISCLOSURES

None of the authors have any relevant disclosures to make. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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