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Thermotolerant coliforms in drinking water as predictors of diarrhea:
An analysis of combined data from multiple studies

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Thermotolerant coliforms in drinking water as predictors of diarrhea: An analysis of combined data from multiple studies

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Abstract

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By James M Hodge

Background: Inadequate access to microbiologically safe water continues to be a driver of the global burden of diarrheal disease. Interventions to improve water quality have been shown to be effective at both improving the microbiological safety of drinking water as well as reducing risk of diarrhea. When evaluating water quality in the field, thermotolerant coliform (TTC) bacteria indicative of fecal contamination are frequently used due to the infeasibility of pathogen specific tests. However, the association between the quantity of TTC in drinking water and health outcomes is not well defined. This study aims to address this knowledge gap and provide further evidence as to whether improving drinking water quality has an impact on risk of diarrhea.

Methods: Individual level data was obtained from seven previous studies that collected data on water quality based on TTC/100ml and self-reported diarrhea over a seven-day recall period. Data was combined into one data set and analyzed using multilevel logistic regression models with diarrhea as a binary outcome variable and TTC/100ml as the predictor variable. Odds ratios were calculated for TTC as a continuous variable to evaluate whether increases in TTC/100ml are associated with an increased risk of diarrhea and as a categorical variable to evaluate whether there is evidence of a dose-response or threshold effect.

Findings: For the combined data set there is a statistically significant association between TTC/100ml and diarrheal disease for all ages (OR: 1.12; 95% CI: 1.08-1.18) and for children under five (OR: 1.18; 95% CI: 1.11-1.26). There was also evidence of both a threshold effect at 10 TTC/100ml when compared to <1 TTC/100ml and a dose-response effect. Odds ratios followed a significant increasing linear trend ($p<0.001$) as the exposure categories increased.

Conclusions: This study found evidence of a significant association between TTC and diarrheal disease as well as significant dose-response and threshold effect. These results challenge recent studies and provide support for health-based WHO guidelines that limit TTC levels in drinking water. Furthermore, the association between fecal contamination and risk of diarrhea found here provides further support to suggest that improving drinking water quality is an important method of reducing the global burden of diarrheal disease.

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Contents

Section 1: Introduction and Rationale

In 2013, diarrheal diseases caused an estimated 1.3 million deaths and were the fourth leading cause of years of life lost in developing countries (GBD 2013, 2015). For children under 5 years of age, diarrheal diseases were the fourth leading cause of death and caused approximately 800 thousand deaths in 2010 (Liu et al., 2012). The majority of these deaths occurred in developing regions where they accounted for 12% of all child deaths in Africa and the Eastern Mediterranean and 11% of all child deaths in Southeast Asia compared to only 4% of all child deaths in the Americas and Europe (Liu et al., 2012). The disparity between developed and developing countries is reflected in the fact that the five largest contributors to deaths by pneumonia and diarrhea were two countries in South Asia (India and Pakistan) and three countries in sub-Saharan Africa (Nigeria, Democratic Republic of Congo, and Ethiopia) (Liu et al., 2012) with more than half of all child deaths due to diarrhea occurring in Africa (World Health Organization, 2009).

One of the main drivers of this global burden is inadequate access to safe water. The link between water contaminated with feces and diarrheal disease has been known at least since John Snow's study of cholera in London in 1854. Increasing knowledge of pathogen transmission through fecally contaminated water in the early 20th century led the US Public Health Service to establish the first bacteriological guidelines for drinking water in 1914 (United States Environmental Protection Agency, 2000). The introduction of microbiological standards and subsequent increasing coverage water treatment systems using filtration and disinfection in the United States have been shown to have contributed to a dramatic reduction in incidence of waterborne illnesses (Cutler & Miller,

2005). The impact historical improvements and the importance placed on clean water is further reflected in drinking water standards and regulations including those of the US EPA (40 CFR 141.63, 2000) and the World Health Organization (WHO) (World Health Organization, 2011) that require that there be no detectable levels of fecal contamination in drinking water. Improvements in provision of safe water are seen as so beneficial that CDC included sanitation and hygiene for control of infectious disease as one of the top ten public health achievements of the 20th century (CDC, 1999) and a reader poll conducted by the British Medical Journal voted clean water and sewage disposal as the most important medical advance since 1840 (Ferriman, 2007).

Despite the clear benefits of improved water quality and recognition of the importance of clean water and sanitation, approximately 700 million people remain without sufficient access to an improved water source (WHO/UNICEF, 2014). Thus, the United Nations included improving access to safe drinking water in the Millennium Development Goals (MDGs) which sought to "halve, by 2015, the proportion of the population without sustainable access to safe drinking water … ." (United Nations, 2013). To achieve this goal, there have been numerous interventions aimed at improving water quality and evidence suggests that these interventions have been effective at both improving the microbiological quality of water and reducing risk of diarrheal disease (Arnold & Colford, 2007; Clasen, Roberts, & Rabie, 2006; Fewtrell, Kaufmann, & Kay, 2005; Wolf et al., 2014).

However, the view that improving water quality in developing countries results in reductions in diarrheal morbidity is not universally held. A systematic review by

Cairncross et al. (2010) found that when only blinded studies were analyzed, improving water quality had no apparent effect on diarrheal morbidity. A second yet-to-be published systematic review by Engell & Lim (2013) found no significantly greater effects of piped water or source water treatment compared with water supply and "no difference in point-of-use interventions when blinding was taken into account (p=0.08)." The Engell & Lim review led the 2010 Global Burden of Disease (GBD) study (Lim et al., 2012) to conclude that the effect of improved drinking water and sanitation may be smaller than previously thought. The conclusions of the Engell & Lim review and the GBD study were significantly different than prior reviews and revived debate over whether interventions to improve water quality provide any measureable health benefit.

One aspect of this debate stems from the method used to measure fecal contamination of water. In many settings where water quality interventions are implemented, it is not feasible to measure multiple pathogens (World Health Organization, 2011). Therefore, water quality is typically assessed by measuring bacteria indicative of fecal contamination, usually either *E. coli* or thermotolerant coliforms (TTC). Thermotolerant coliforms (sometimes referred to as fecal coliforms) are a class of bacteria consisting of four species of coliforms that grow at elevated temperatures (44.5 ± 0.2° C): *Escherichia coli, Klebisella*, *Enterobacter*, and *Citrobacter* with *E. coli* being the predominant species (Tallon, Magajna, Lofranco, & Leung, 2005). TTC were used as indicators of fecal contamination because it was thought that the elevated temperature required for their growth precluded environmental presence except where there was fecal contamination (Ashbolt, Grabow, & Snozzi, 2001). But, each type of TTC and *E. coli*

have been found in the environment in the absence of fecal contamination (Ashbolt et al., 2001; Rivera, Hazen, & Toranzos, 1988; Toranzos, 1991). Furthermore, although TTC and *E. coli* are indicators of fecal contamination, they are not well correlated with the presence of pathogens (Wu, Long, Das, & Dorner, 2011) and, as discussed further below, the association between indicator bacteria and health risk is inconclusive. Nevertheless, WHO authorizes the use of *E. coli* or TTC for use as indicators of fecal contamination and states that neither should be detectable in treated drinking water (World Health Organization, 2011).

Because TTC are not pathogenic, but are merely indicators of contamination, the relation of levels of TTC in drinking water to health outcomes is unclear. Previous studies that assessed the association between *E. coli* or TTC and diarrhea as the primary outcome have been inconclusive with only two finding an association with *E. coli* (Brown, Proum, & Sobsey, 2008; Levy, Nelson, Hubbard, & Eisenberg, 2012), one finding an association only above a certain threshold level (Moe & Sobsey, 1991) and the remainder finding no association for either TTC or *E. coli* (Han, Oo, Aye, & Hlaing, 1991; Henry & Rahim, 1990; Jensen, Jayasinghe, van der Hoek, Cairncross, & Dalsgaard, 2004; Knight et al., 1992). However, several of these studies indicated that sample size and statistical power might have been insufficient to see any effect (Han et al., 1991; Henry & Rahim, 1990; Jensen et al., 2004; Knight et al., 1992; Levy et al., 2012). Several studies have also explored the association between indicator bacteria and diarrheal disease as a secondary outcome within larger studies and reached differing conclusions. Aceituno & Stauber (2012) found an association between *E. coli* and diarrheal disease and Clasen et al (2005) and Peletz et

al. (2012) found evidence of an association between TTC and diarrheal disease; a larger study by Boisson et al. (2013) in India found no association.

Two systematic reviews have also been conducted and reached differing results. Gundry et al. (2003), conducted a review and meta-analysis of studies that used either *E. coli* or TTC as the indicator bacteria and assessed risk of diarrheal disease based on the level of contamination and found that there was no significant association between *E. coli* and TTC and diarrheal disease. In 2014, Gruber et al. (2014), conducted an updated review that included more studies that used *E. coli* as the indicator bacteria. The additional studies allowed them to assess *E. coli* and TTC separately where Gundry, et al. had assessed all indicators combined. In their pooled analysis, Gruber et al. (2014) found that when combined, the indicator bacteria were not associated with diarrheal disease (RR: 1.26; 95% CI: 0.98-1.63), but when analyzed separately, *E. coli* was associated with an increased risk of diarrheal disease (RR: 1.54; 95% CI: 1.37-1.74) but TTC were not (RR: 1.07; 95% CI: 0.79-1.45).

Significantly, both the Gundry et al. (2003) study and the Gruber et al. (2014) study extracted summary estimates from previous studies to estimate pooled risk ratios; they did not conduct any analyses using individual level data.

We sought to further explore the relation between TTC levels in drinking water and diarrheal disease. However, instead of conducting another meta-analysis of pooled estimates, we combined and analyzed individual level data on water quality and recent diarrhea from multiple studies. To ensure comparability of data across studies, we only included studies that met specific inclusion criteria with respect to how water samples

were collected and assayed and how diarrheal disease was measured. Though none of the studies from which data was obtained were designed to specifically address this question, by aggregating the data from these studies that used consistent methodologies, we were able to conduct a secondary analysis of individual health outcomes linked to a specific household drinking water sample. This method allowed an analysis of whether and to what degree drinking water quality effects health risk across a broad range of countries and settings.

Section 2 Literature Review

Global burden of diarrheal disease

In 2013, diarrheal diseases caused an estimated 1.3 million deaths and were the fourth leading cause of years of life lost in developing countries (GBD 2013, 2015). For children under 5 years of age, diarrheal diseases were the fourth leading cause of death and caused approximately 800 thousand deaths in 2010 (Liu et al., 2012). The majority of these deaths occurred in developing regions where they accounted for 12% of all child deaths in Africa and the Eastern Mediterranean and 11% of all child deaths in Southeast Asia compared to only 4% of all child deaths in the Americas and Europe (Liu et al., 2012). The disparity between developed and developing countries is reflected in the fact that the five largest contributors to deaths by diarrhea were two countries in South Asia (India and Pakistan) and three countries in sub-Saharan Africa (Nigeria, Democratic Republic of Congo, and Ethiopia) (Liu et al., 2012) with more than half of all child deaths due to diarrhea occurring in Africa (World Health Organization, 2009).

Inadequate access to safe water

One of the primary drivers of the burden of diarrheal diseases in the developing world is inadequate access to safe water. It is well established that providing microbiologically safe water can lead to substantial positive impacts on public health. For example, Cutler and Miller reviewed evidence of the impact of improved water treatment systems in thirteen cities in the United States in the early 20th century (Cutler & Miller, 2005). They found that the introduction of chlorination and filtration systems explained nearly half of the reduction in mortality between 1900 and 1936 and concluded that introduction of technologies to treat water supplies were "likely the most important public health intervention of the twentieth century." (Cutler & Miller, 2005). More recently, a systematic review of water treatment interventions by Wolf et al. found that changing from using an unimproved water source to piped water of high quality led to an 81% reduction of diarrheal disease risk (79% when adjusted for non-blinding) (Wolf et al., 2014). Nevertheless, access to safe drinking water remains a problem particularly in developing countries.

Global recognition of the problem was sufficient that it was included in the Millennium Development Goals (MDG) as Target 7.C which aimed to halve the proportion of the population without sustainable access to safe drinking water and basic sanitation (United Nations, 2013). The World Health Organization and UNICEF track the progress on this goal in their Joint Monitoring Programme for Water Supply and Sanitation (JMP) which assesses the number of people using "improved" sources which include piped water, public tap/standpost, tubewell/borehole, protected dug well, protected spring,

and rainwater (WHO/UNICEF, 2014). Although significant progress has been made – in fact 116 countries have met the target for water – an estimated 700 million people still lack access to an improved water supply (WHO/UNICEF, 2014). Nearly half of those that lack access to an improved water supply live in sub-Saharan Africa despite nearly a quarter of that population gaining access to an improved drinking water source since 2000 (WHO/UNICEF, 2014).

Furthermore, even those that have access to an improved water supply may not have access to a source of drinking water that is microbiologically safe (Bain, Cronk, Wright, et al., 2014; Onda, LoBuglio, & Bartram, 2012). The WHO defines safe drinking water as water that "does not represent any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages." (World Health Organization, 2011). According to WHO, water for consumption should contain no fecal indicator organisms (World Health Organization, 2011). However, a study by Brown et al. (2013) in Vietnam found that approximately 51% samples from improved water sources were contaminated by more than 10 colony forming units (CFUs) of *E. coli* fecal indicator bacteria per 100 ml. A similar study in Cambodia by Shaheed et al. (2014) reached the same conclusion stating that "improved" drinking water sources may be unsafe in some settings particularly when there is a high amount of recontamination during water storage. A recent systematic review also looked at this issue and found that improved sources were significantly less likely to be contaminated but also noted that in 38% of 191 studies, indicator bacteria exceeded WHO safe levels in at least 25% of the samples (Bain, Cronk, Wright, et al., 2014). In a separate review, Bain, et al. (2014)

estimated that 1.3 billion people continue to get their drinking water from an unimproved source or a source with ten or more fecal indicator bacteria per 100 ml. Thus, even though significant improvements have been made to increase access to an improved water source, there remains ample room for improving the accessibility of microbiologically safe drinking water.

An additional concern with relying on improved source water alone is the evidence that drinking water is subject to recontamination while being stored in the home (Clasen & Bastable, 2003; Levy, Nelson, Hubbard, & Eisenberg, 2008; Wright, Gundry, & Conroy, 2004). The lack of sufficiently safe water and the problem of recontamination has led to an increasing emphasis on point-of-use or household water treatment and safe storage including recognition by the WHO that when there is no reliable safe water supply, "tools and education should be made available to implement individual or household-level treatment and safe storage." (World Health Organization, 2011).

Household water treatment has also been shown to be effective at reducing the risk of diarrheal illnesses. Systematic reviews have consistently found household water treatment to reduce risk of diarrheal illnesses though recent refined efforts have begun to distinguish between treatment methods and adjust for study quality. In 2005, Fewtrell et al. (2005) reviewed 12 studies of household water treatment found that it showed a 35% reduction in relative risk (95% CI: 12-52%). In 2006, Clasen et al. (2006) conducted a larger review of 30 studies with 38 discrete comparisons. Pooled analyses for different effect measures were reported separately but found between 35-51% reductions in diarrheal risk among all ages. In 2007, Arnold et al. (2007) conduced a systematic review

of chlorination interventions calculated a pooled reduction in risk of 29% (95% CI: 13-42%) for the ten studies analyzed.

In 2010, Cairncross et al. (2010), conducted a systematic review of water interventions that also assessed the difference between blinded and open trials. They found that when only blinded studies were assessed the reduction in risk of diarrhea was only 7% and was not statistically significant leading to the conclusion that existing data from trials and reviews based on them did not offer "a firm basis for judging the effect of water quality improvements." (Cairncross et al., 2010). This conclusion was reassessed by a yet-to-be published systematic review (Engell & Lim, 2013) and adopted by the Institute for Health Metrics and Evaluation in its Global Burden of Disease analysis (Lim et al., 2012). An abstract for the Engell & Lim (2013) study states that they found no difference in diarrhea from point of use interventions when blinding was taken into account and concluded that water quality interventions have a much smaller impact than previously thought.

The conclusions of the Cairncross, et al. review and the Engell & Lim review were significantly different than results and conclusion from previous analyses and led to an update of previous reviews. Wolf et al. (2014) found a protective effect while using a different method of analysis. They grouped studies by baseline water source as well as treatment method and found protective effects for each comparison except when the intervention was chlorination and the baseline water source was basic piped water. For household treatment methods (excluding piped water), the transition from an unimproved source to a filter with safe storage had the largest effect with a 59% reduction

in risk (95% CI 50-67%). Crucially, Wolf et al. (2014) also adjusted for non-blinding and in that analysis, filters and safe storage continued to show a significant reduction when the baseline water source was unimproved or an improved community source (45% and 38%, respectively).

Indicator bacteria

Whether improving access to a safe source or providing a means of household treatment, when assessing whether water quality interventions are effective at improving water quality, fecal indicator bacteria are used as a proxy for pathogens. The most commonly used fecal indicator bacteria are from the coliform group and they have been used to measure water quality since the late 19th century (Tallon et al., 2005). Currently, thermotolerant (fecal) coliforms and *Escherichia coli* are the most commonly measured indicator bacteria (Garcia-Armisen, Prats, & Servais, 2007). The WHO recommends their use in its drinking water guidelines and states that "water intended for human consumption should contain no fecal indicator organisms." (World Health Organization, 2011).

Total coliform bacteria are a large group of "aerobic and facultatively anaerobic, Gram negative, non-spore forming bacilli" (World Health Organization, 2011). Coliform bacteria that are able to ferment lactose at 44-45° C are known as thermotolerant coliforms (TTC) generally predominated by *E. coli* (World Health Organization, 2011). *E. coli* is present in high numbers in human and animal feces and is rarely found in the absence of fecal pollution (World Health Organization, 2011). TTC are less specific and can include some environmental organisms (World Health Organization, 2011). However,

E. coli has been estimated to be approximately 94% of the TTC in human feces (Tallon et al., 2005). Additional studies by Garcia-Armisen et al. (2007) in France and Hachich et al. (2012) in Brazil found that *E. coli* were 77% and 84.3% of TTC isolated from fresh water respectively.

Despite widespread use as indicators of fecal contamination, TTC and *E. coli* do not correlate well with the presence of pathogens. A 2011 review by Wu et al. (2011) analyzed 540 pathogen-indicator comparisons and found that no one indicator was most likely to be correlated with a pathogen. Furthermore, there is evidence that TTC and *E. coli* can survive in the environment (Rivera et al., 1988; Solo-Gabriele, Wolfert, Desmarais, & Palmer, 2000).

Indicator bacteria and diarrheal disease

Because of their common usage as a measurement of water quality and inclusion as main monitoring organisms in the WHO guidelines, research has been conducted to explore whether indicator bacteria are related to diarrheal disease. Henry and Rahim (1990) studied the relationship between diarrhea and TTC in drinking water in Bangladesh within a study of the effect of sanitation and hygiene on diarrheal disease. The authors found high levels of water contamination among households with improved and unimproved sanitation with 35.7% and 58% of water samples having greater than 10,000 TTC/g. However, when comparing diarrhea between low (<10,000 TTC/g) and high (>10,000 TTC/g) exposure groups the authors found no association for either unimproved (RR: 0.86; 95% CI: 0.34-2.18) or improved (RR: 2.58; 0.70-9.54) sanitation (Henry & Rahim, 1990). A study by Moe & Sobsey in 1991 in the Philippines assessed the relation between

E. coli and TTC (as well as fecal *Streptococci* and *Enterococci*) in source water and diarrheal disease (Moe & Sobsey, 1991). Each indicator showed a positive association between exposure and risk of diarrhea though only *E. coli* and *Enterococci* were statistically significant (Moe & Sobsey, 1991). Significantly, a dose-response and threshold analysis was also conducted and found that contamination of greater than 1000 CFU/100ml of water was related to diarrheal disease for each indicator with *E. coli* being a "strongly significant predictor" while TTC were "marginally significant" (Moe & Sobsey, 1991, p. 312).

Several studies since Moe, et al. have also looked at whether exposure to TTC is associated with diarrheal disease and reached differing results. Han, et al. (1991) conducted a study in Myanmar assessing the association between contaminated food and water and incidence of diarrhea as the primary objective. They categorized water contamination as low (0 TTC/100ml), medium (geometric mean: 1.3 TTC/100ml), and high (geometric mean: 3.4 TTC/100ml). No association was found when water that was either medium (RR: 0.73; 95% CI: 0.52-1.03) or highly (RR: 0.72; 95% CI: 0.51-1.01) contaminated with TTC was compared with water that did not contain TTC though this study was only powered to detect a relative risk of 2, samples were only collected of the food and water given to children in the morning, only 22% of water samples were positive for TTC, the geometric mean TTC/100ml was very low (11.5; SD: 4.5), and the categories of contamination had very small ranges. Knight, et al., (1992) conducted a case-control study assessing risk factors for diarrhea including water quality measured by TTC. They analyzed presence/absence of TTC and found that absence of TTC in both source (OR:

0.77; 95% CI : 0.39-1.50) and drinking water (OR: 0.69; 95% CI: 0.21-1.75) was not significantly protective against diarrhea but only 12% of water samples were contaminated (Knight et al., 1992).

The remainder of studies that have assessed the relationship between TTC and diarrhea have done so as a secondary objective in the context of larger randomized controlled trials (RCT) of household water treatment interventions. In 2005, Clasen et al. (2005) assessed the relation between TTC and diarrheal disease as a secondary objective of an RCT of a household water treatment intervention in Colombia and found that odds of diarrhea increased with a log 10 increase in TTC for all ages (OR: 1.48; 95% CI 1.12-1.95) and children under 5 (OR: 1.47; 95% CI 1.01-2.15). As a secondary objective of a RCT of a household water filter intervention, Peletz et al. (2012) reported an increase in longitudinal prevalence with each log 10 increase in TTC/100ml for all ages (LPR: 1.29; 95% CI: 1.14-1.45) and for children <2 (LPR: 1.20; 95% CI: 1.05-1.39) and a positive linear trend for probability of diarrhea as the level of TTC/100ml increased suggesting a dose-response effect. Most recently, Boisson et al. (2013) reported in an RCT of water chlorination from India, that they found no association between TTC levels and increased risk of diarrhea for children under 5 drinking water with >1000 TTC/100ml compared to those drinking water with <1000 TTC/100ml (LPR: 1.12; 95% CI: 0.84-1.49) or among participants of all ages

Two recent systematic reviews have addressed whether indicators are related to diarrheal disease and reached differing results. Unlike the studies discussed above and the current study, these reviews conducted a meta-analysis using the reported results

extracted from previous studies and did not do any further analysis of individual level patient data. The first by Gundry et al. was published in 2004 and reviewed studies that compared indicator counts in stored water with diarrheal diseases (Gundry et al., 2003). They included observational or intervention studies of any age group where water is transported to and stored in the home from an outside source and assessed diarrhea or cholera as the health outcome. For observational studies, odds ratios were extracted for high quality water compared to low quality water regardless whether the indicator bacteria studied was *E. coli* or TTC. For intervention studies, the reported odds ratios were extracted when possible and otherwise calculated from the reported number of subjects and cases in the intervention and control groups. Twelve studies met the inclusion criteria and were included in the analysis: 3 that used *E. coli*, 8 that used TTC, and 1 that used both (Gundry et al., 2003). All but one of the studies assessed stored water quality and general diarrhea (Gundry et al., 2003). A pooled odds ratio was calculated using random effects models. The estimated pooled odds ratio for POU water quality for both *E. coli* and TTC combined was 1.12 (95% CI 0.85-1.48) which suggests that there is no relation between indicator bacteria and diarrheal disease (Gundry et al., 2003).

In 2014, Gruber, et al. (2014) updated the previous review and included new studies looking at the relation between *E. coli* and diarrheal disease which allowed for separate analyses of *E. coli* and TTC and diarrheal disease. They included studies that collected water quality and health outcome data at the household or point-of-use level and did not exclude studies unless they only reported source water quality, used ecological level health data. Definition of diarrhea, recall period, study design, age

groups, study location, study setting, and drinking water source were not considered when determining whether to include a study. When possible, reported relative risks were extracted and used for the analysis. Where no measure of risk was reported, the authors used raw data to calculate effect measures and confidence intervals "using standard methods." (Gruber et al., 2014). A meta-analysis was conducted using random effects models and inverse variance weighting to estimate a summary effect measure using the "the lowest extractable threshold" from each study (Gruber et al., 2014). The combined meta-analysis of *E. coli* and TTC studies reached a similar conclusion and Gundry, et al. (2003) finding a positive but not statistically significant association between the indicator bacteria and diarrheal disease (RR: 1.26; 95% CI: 0.98-1.63) (Gruber et al., 2014). However, when *E. coli* and TTC study results were separately analyzed, the results differed. They found that *E. coli* studies consistently reported a positive association and the pooled analysis suggested that increased concentrations are associated with increased risk of diarrheal disease (RR: 1.54; 95% CI: 1.37-1.74) (Gruber et al., 2014). However, studies that used TTC did not show a similar effect suggesting that there is no association between TTC and risk of diarrheal disease (RR: 1.07; 95% CI: 0.79-1.45) (Gruber et al., 2014). The analysis of a dose-response and threshold level found no evidence of either but both were limited due to a low number of studies that reported such analysis (Gruber et al., 2014).

The possible environmental persistence combined with the weak evidence suggesting that indicators are related to health outcomes has led some researchers to suggest using indicators as a proxy for health risk cautiously (Levy et al., 2012). Levy et al.

(2012) explored the relationship between *Enterococci*, *E. coli*, and somatic coliphages in source water and household drinking water and diarrhea in Ecuador. They conducted five separate assays (3 for *E. coli* and 1 for Enterococci and somatic coliphages) and found that only *E. coli* in household samples and samples of combined household and source water measured by mI agar showed a significant increased risk of diarrhea for a one order of magnitude increase. They also found no evidence of a dose-response or threshold effect. However, they are careful to note that their analyses might have been limited by sample size with too few cases of diarrhea and household water samples to detect an effect. Nevertheless, the apparent lack of an association in their analysis led them to conclude that indicators as a proxy for health risk should be used cautiously (Levy et al., 2012). Despite these drawbacks, the WHO currently recommends *E. coli* as an indicator bacteria with TTC as an acceptable alternative for monitoring of fecal contamination of drinking water (World Health Organization, 2011).

This thesis provides further evidence as to whether TTC indicator bacteria in drinking water are associated with diarrheal diseases. We aggregated individual patient level data from seven studies that used a similar approach to assess diarrheal disease and water quality (sampling drinking water at the time of the visit and assaying the same for TTC). The studies all used the WHO definition of diarrhea of three or more loose or watery stools in a 24-hour period (World Health Organization, 2005) and each study measured the level of TTC using a standard membrane filtration method (APHA, AWWA, & WEF, 2005). Significantly, none of the studies was designed to assess the link between water quality and diarrhea. Rather, they were RCT or cross-sectional studies seeking to evaluate

the health impact interventions to improve water quality or sanitation interventions with which TTC of drinking water was collected simply to assess the impact of the intervention on exposure. Thus, as separate studies, they may not have been powered to assess the link between water quality and diarrhea. Aggregating individual patient level data from all the studies in this secondary analysis provides an opportunity to investigate this question.

Section 3: Methods

Included studies

Individual level data was drawn from 7 studies of water quality and sanitation conditions and diarrheal disease. The studies included in this analysis were chosen because data was available and because the studies used the same method for collection and analysis of water samples, the same definition of diarrheal disease (3 or more loose or water stools in a 24-hour period) and the same method to ascertain cases of diarrheal disease (self-reported cases with a 7 day recall period) for both children <5 years and for householders of all ages. Significantly, none of the studies was designed or powered to investigate an association between water quality and diarrhea, though in some cases the researchers explored whether there was evidence of such an association in the particular study.

Five of the studies were randomized controlled trials of household water treatment interventions (Boisson et al., 2010; Boisson, Schmidt, Berhanu, Gezahegn, & Clasen, 2009; Clasen, Brown, & Collin, 2006; Clasen et al., 2005; Peletz et al., 2012). One study was a randomized controlled trial of a sanitation intervention (Clasen et al., 2014).

One study followed a cross-sectional design (Peletz et al., 2011). One of the randomized controlled trials (Boisson et al., 2009) did not include usable water quality data for the follow-up time period, so only baseline measurements were used and it was treated as a cross-sectional study. All were conducted among rural, low-income populations, except for Zambia which was a peri-urban setting where the study population was limited to households with children <2 years whose mothers with HIV+. For the randomized controlled trials, data from both the intervention and control groups was used. Table 1 provides more details on the individual studies used for this analysis.

Methods for assessing diarrhea and water quality

In each study, diarrheal prevalence was obtained during the same household visit at which the water samples were collected. During the household visit, diarrhea prevalence over the preceding seven days was ascertained by asking the female head of household or primary caretaker if any household members had had diarrhea during the past seven days. In each study diarrhea was defined as 3 or more loose or water stools in a 24-hour period (World Health Organization, 2005).

On the same visit to obtain diarrheal prevalence, researchers obtained a water sample by asking the female head of household what water was being used by householders at that time for drinking. Water samples were collected from stored household drinking water during household visits in either sterile 125ml Nalgene bottles (Nalge Nunc International, Rochester, NY) or sterile 125ml WhirlPak bags (Nasco International, Fort Atkinson, WI) containing a sodium thiosulfate tablet to neutralize any chlorine. All samples were stored on ice during transport and were processed within four

hours assess TTC levels. Microbiological assays were done using a standard membrane filtration method (APHA, AWWA, & WEF, 2005) with membrane lauryl sulphate medium. Samples were incubated at 44 ± 0.5° C for 18 hours in a DelAgua portable incubator (Robens Institute, University of Surrey, Gilford, Surrey, United Kingdom). Following incubation, the number of colonies were counted and recorded as individual TTC and standardized to a count of TTC/100ml of water.

Data extraction and synthesis

Original data were obtained from the researchers of the previous studies for each surveillance visit. Diarrheal prevalence was obtained for individual householders; water quality data was obtained for the household and ascribed to each member of that household for that visit. The data were combined into one data set retaining variables for age, household, and study location for each individual as well as whether the individual reported having diarrhea over the preceding seven days at each follow-up round. Household level data on water quality (measured as colony forming units (CFU) of TTC per 100ml of water) at each follow-up round was retained and matched to all individuals within a household. Because diarrhea generally varies over seasons (Das et al., 2014; Fisman, 2007; Levy, Hubbard, & Eisenberg, 2008), an additional variable for the season (rainy/dry) was also included. Season was only recorded for two of the studies (Peletz et al., 2011, 2012). For the remainder, the season variable was assigned based on the date at which the observation occurred and data on rainfall from the National Climatic Data Center (National Climatic Data Center, 2015) or Weatherbase (Weatherbase, 2015).

Statistical Analysis

Once the data were cleaned and aggregated into a complete working data set, analysis was done using a multi-level mixed effects model. Random effects were included to account for repeated measurements in individuals and clustering at the household level. Water quality was included as the predictor variable as log 10 transformed CFU/100ml. The dependent variable was diarrheal disease as a binary (yes/no) outcome.

The relationship between TTC and diarrheal disease was assessed separately for each study and again for the combined data set. Two models were fitted for each study for all ages and again for children under five years of age only. The first model was used to assess whether there was an apparent relationship between the number of TTC/100ml and odds of diarrhea. It modeled log-odds of diarrheal disease using log 10 TTC/100ml as a continuous predictor variable to evaluate the odds of diarrhea for each log 10 increase in TTC. To assess whether a dose-response or threshold level of TTC was evident, the second model was fitted using WHO risk categories (World Health Organization, 1997) for five levels of contamination: <1 TTC/100ml, 1-10 TTC/100ml, 11-100 TTC/100ml, 101-1000 TTC/100ml, and >1000 TTC/100ml. Adjusted odds ratios were calculated for each category using <1 CFU/100mL as the reference group. Models that included all ages were controlled for age by including it as a categorical variable (<5, 5-15, >15) while the models limited to children under five years of age included age as a continuous variable. Season was controlled for in all models except those for the Chiñiri study (Clasen, Brown, et al., 2006) which was conducted entirely within one season. Models fitted using the combined data set were also adjusted for study location. The resulting odds ratios were qualitatively

evaluated for evidence of a threshold and a linear trend test was conducted to assess if there was a dose-response.

Sensitivity Analysis

A sensitivity analysis was also conducted to qualitatively assess the effect of the Indian sanitation study (Clasen et al., 2014) which contributed the majority of the observations for this analysis. Each model was fitted using the combined data set with the exception of the data from the Indian sanitation study to determine the extent to which the overall outcome was influenced by that study. All data cleaning and management was done using SAS 9.4 (SAS Corporation, Cary, NC) and all models were fitted using Stata 13 (StataCorp, College Station, Texas). Graphics were generated using R version 3.1.2.

Ethics

The protocol for this study was approved by the Emory University Institutional Review Board (IRB00079426). Each of the studies from which data was obtained was approved by the Ethics Committee of the London School of Hygiene and Tropical Medicine and by local ethics committees in the countries in which they were conducted.

Section 4. Results

Population and demographics:

Table 2 shows the distribution of observations among the studies as well as age distributions and observations per season for each study individually and overall. The combined data set included data for 4,017 households and 26,518 individuals. The Indian sanitation trial contributed the majority of the households (72.2%) and individuals

(79.3%). The Indian sanitation trial had an older population as well with a mean age of 26.8 years. The mean age of individuals for the combined data from all studies was 26.47 years. The Zambia RCT had the lowest mean age of 14.76 years. Distribution among the age categories was variable between studies as well with the Zambia RCT having the highest proportion of children under five (34%) and the Ethiopia RCT having the lowest (12.9%).

Diarrhea prevalence

Baseline prevalence among the individual studies also varied with the Colombia RCT having the highest at 21.77% of respondents reporting having had diarrhea in the preceding seven days. Mean prevalence for the follow-up periods ranged from 2.33% in the Zambia RCT to 7.3% for the Colombia RCT. The mean prevalence for the combined data is 3.73%. Table 3 shows the distribution of diarrhea for each study by age category, TTC category, and season as well as the overall prevalence of diarrhea in each category. In all studies, children under five had the highest prevalence of diarrhea over the length of the studies and for the combined data, prevalence among children under five was 10.9%. Prevalence among categories of TTC were variable between studies but in general, prevalence increased with increasing TTC loads. In the combined data, prevalence increased from 3.9% for <1 TTC/100mL to 5.2% for >1000 TTC/100mL. Prevalence of diarrhea cases by season was variable across studies and in part reflected the distribution of observations among seasons. However, when combined, there was a significant ($p<0.001$) difference in prevalence of cases in the rainy and dry seasons.

Water quality

Table 4 shows water quality data for each study separately and for the combined data. Water quality was highly variable across and within studies. The DR Congo study had the highest arithmetic mean with 1548.45 TTC/100ml. Chiñiri had the lowest arithmetic mean with 35.26 TTC/100ml. All of the studies had highly skewed TTC data as seen by the difference between the mean and median values. Thus, values of TTC were log 10 transformed prior to analysis. The majority of studies had high numbers of households with <1 TTC/100ml. This result is not unexpected considering four of the five studies were assessing water treatment technologies and the data includes households in both the intervention and control arms of the studies. However, all the studies also had at least 10% of the observations in the highest category. For the combined data set, 30.9% of samples had <1 TTC/100 ml and 17.2% were >1000 TTC/100ml.

TTC-Diarrhea assessment

The combined data set showed that an increase in TTC results in increased odds of diarrhea for all ages and for children under five. Table 5 and Figure 1 show adjusted odds ratios for a one log increase in TTC/100mL of household drinking water. Each of the studies except the Zambia cross-sectional study, the Chiñiri study, and the India sanitation study are significant at an alpha of 0.05 indicating that increasing the level of TTC in drinking water is associated with an increase the odds of diarrhea. For children under five, the adjusted odds ratios are significant at an alpha of 0.05 for all studies except the Zambia and Ethiopia cross-sectional studies and the Chiñiri study. When combined, there is a 12% (95% CI: 8-18%) greater odds of diarrhea for each one log increase in TTC/100ml

for all ages. For children under five the effect is larger with an 18% (95% CI: 11-26%) increase in odds of diarrhea for each log increase in TTC/100ml. These results indicate that there is a statistically significant association between the level of contamination with TTC and diarrhea prevalence. They also show that a higher level of contamination corresponds with higher odds of having had diarrhea over the preceding days, especially among children under five years old.

Threshold effect and dose-response assessment

Table 6 shows the adjusted odds ratios for the increasing categories of TTC/100ml with the lowest category (<1 TTC/100ml) as the reference category. In each study and in the combined analysis, there is a positive relationship between the higher categories and odds of diarrhea in the past seven days for all ages and for children under five years old. Furthermore, and perhaps most importantly, the combined data shows evidence of increased odds of diarrhea at a threshold level of 10 TTC/100ml. For all ages, there is a small but non-significant positive effect with the odds ratio of diarrhea in the preceding seven days for the 1 to 10 TTC category compared to the <1 category is 1.02 (95% CI: 0.82-1.26). For children under five, the odds ratio is similarly non-significant: 0.94 (95% CI: 0.68-1.31). However, for the 11 to 100 TTC category and for all higher categories, for all ages and for children under five years old, the odds ratios show a positive association and are all significant at an alpha of 0.05 (Figure 2).

Sensitivity analysis

The Indian sanitation trial contributed the majority of the observations to the combined data set and it had a clear effect on the overall outcome. The adjusted odds

ratio for all ages for the Indian sanitation study was positive but not significant (OR: 1.04, 95% CI: 0.99-1.10) while the adjusted odds ratio for the combined data excluding the Indian sanitation trial showed a greater effect that is statistically significant (OR: 1.36, 95% CI: 1.26-1.47). Thus, it is not surprising that the adjusted odds ratio all the data is closer to unity (OR: 1.12, 95% CI: 1.08-1.18). However, though the odds ratio is lower, the positive association remained significant and does not change the conclusion that there is an association between increasing log10 levels of TTC in drinking water and increased odds or diarrhea over the preceding seven days.

Section 5. Discussion

The purpose of this study w to combine data from multiple studies to assess the relationship between TTC bacteria and diarrhea, to explore whether there is a dose-response relationship and to determine whether there is a threshold level of TTC that is not associated with diarrhea. Currently, TTC are considered an acceptable alternative for *E. coli* as a measure of fecal contamination of drinking water and possible presence of pathogens (World Health Organization, 2011). However, questions have been raised about the association between water quality and diarrhea based on recent reviews of blinded trials and meta-analyses of other studies.

Our analysis of the combined data from 7 comparable studies showed a significant increase in odds of diarrhea with increasing log 10 TTC in drinking water (OR: 1.12, 95% CI: 1.08-1.18). The observed effect was stronger for children under five (OR: 1.18, 95% CI: 1.11-1.26) and was even greater when the largest study was excluded from the analysis (OR: 1.36, 95% CI: 1.26-1.47) (Table 5).

This suggestion of an association is further supported when the data was analyzed by WHO risk category (World Health Organization, 1997). For the combined data, the highest risk level (>1000 CFU/100ml) had the highest odds ratio for all ages (OR: 1.49, 95% CI: 1.27-1.76) and for children under five (OR: 1.77, 95% CI: 1.40-2.24) when compared with <1 TTC/100ml as the reference group. Furthermore, the significant linear trend observed for the combined data set provides further evidence that risk increases as the level of contamination increases suggesting that there is a dose-response effect.

The analysis of TTC by WHO risk category also provides evidence of a threshold level. The combined data and the combined data without the India study both show significantly elevated odds ratios beginning at the 11 to 100 CFU/100ml risk category indicating that the threshold level at which the odds of diarrhea becomes significant is >10 CFU/100ml (Table 6). In other words, if household drinking water has 10 or fewer TTC/100ml, odds of diarrhea is not significantly higher compared to household drinking water with <1 TTC/100ml. However, once the number of TTC/100ml surpasses 10, there is a significantly higher odds of having had diarrhea in the previous seven days when compared to water with <1 TTC/100ml. This result differs from that found by Boisson and colleagues (2013) in a similar analysis which found no apparent association among children under five or for all ages comparing exposure levels of >1000 TTC/100ml with <1000 TTC/100ml though that study used a different outcome measure (longitudinal prevalence ratio) and a different recall period for diarrhea (3 days). It likewise differs from Moe & Sobsey (1991) which found an adverse effect only at levels >1000 CFU/100ml for *E. coli* and no indication of a threshold for TTC. Thus, the current results are unique in

showing a clearly defined threshold level for TTC in household drinking water.

Contrary to the conclusion drawn by Gruber and colleagues (2014), these results indicate that there is an association between increasing levels of TTC in drinking water and increased risk of diarrhea. One possible for the difference in findings may be the different methodology used. Though we also drew data from multiple previous studies, we combined and analyzed individual level data allowed for analysis of the effect of TTC on diarrhea at the individual level rather than analyzing the effect estimates to reach a single summary effect. Because the majority of the previous studies found no significant association, it is not surprising that the summary estimate calculated in the systematic reviews was also not significant. However, most of the studies used in the meta-analyses of Gundry et al. (2003) and Gruber et al. (2014) were admittedly limited by sample size. In contrast, this study utilized the individual level data from multiple studies to construct a data set that yielded over 45,000 observations for the combined analysis. The results of the individual studies was similar to that found by those included in the Gundry et al. (2003) and Gruber et al. (2014) reviews in that they showed positive but mostly non-significant associations (Table 5) but the combined the effect becomes significant.

This study is subject to the same limitations as many studies of water quality and diarrheal disease. First, multiple studies have shown that the recall period can be influential on the accuracy of self-reported diarrhea particularly if the recall period is greater than one week (Alam, Henry, & Rahaman, 1989; Arnold et al., 2013; Boerma, Black, Sommerfelt, Rutstein, & Bicego, 1991; Byass & Hanlon, 1994; Feikin et al., 2010). To attempt to minimize this limitation we used only studies that used a uniform seven

day recall period for self-reported diarrhea that has been recommended by some researchers (Arnold et al., 2013). Second, the time lag inherent in measuring water quality and seven day recall of diarrhea means that the drinking water measured is unlikely to be the same drinking water present in the home on the days when diarrhea occurred. Furthermore, the study design in which water samples were collected at the same time that seven-day diarrheal prevalence is recorded does not allow for ascertainment that the source water or water treatment practices were not changed because of the diarrheal disease which could lead to the water sample taken during the household visit to be less contaminated than the drinking water at the time a household member had diarrhea. Finally, the different studies used different values when assigning quantities when the CFU were too numerous to count. However, the values assigned are typically lower than the actual likely value essentially placing a cap on the category that the water quality measurement can fall within. This cap means that there is potential exposure misclassification with high contamination (>1000 TTC/100ml) classified as moderate contamination (101-1000 TTC/100ml).

Despite these limitations, the results of this study suggest that there is an association between the quantity of TTC in drinking water and the odds of diarrhea over the previous seven days. The increasing odds with increasing levels of TTC also provide evidence of a dose-response relationship and the significance of the odds ratios at >10 TTC/100ml indicate that there is a threshold level. Contrary to the GBD study (Lim et al., 2012) and Engell & Lim (2013) review, these results support the conclusion of Wolf et al. (2014) and the reviews beforehand (Clasen, Roberts, et al., 2006; Fewtrell et al., 2005)

that improving water quality by reducing fecal contamination or the amount of fecal contaminants does reduce the risk of diarrheal disease.

Section 6: Conclusion and Recommendations

The purpose of this study was to investigate the relationship between TTC and diarrheal disease to determine if there is any dose-response or threshold level of TTC at which the likelihood of diarrhea is significantly elevated. The results discussed above provide evidence of both a dose response and threshold level of TTC. Few of the previous analyses of this relationship have found significant evidence of an association between TTC and diarrhea. Further, the two meta-analyses found no evidence to suggest that increasing levels of TTC were associated with increasing risk of diarrhea (Gruber et al., 2014; Gundry et al., 2003), leading to a conclusion that only *E. coli* should be used as an indicator of fecal contamination. The results of this study point toward a different conclusion.

In combination, the evidence of a dose-response and threshold found here support the continued use of TTC as an indicator bacteria for fecal contamination. The purpose of fecal indicators is to determine whether there is risk of adverse health impacts from fecal pathogens via a proxy that is more easily measured. On this evidence, there is a clear relationship between TTC and risk of diarrhea. Thus, TTC are fulfilling the purpose of fecal indicator bacteria and in situations where it is infeasible or impossible to measure specific pathogens, measuring TTC provides a reasonable measure of health risk.

Furthermore, the apparent threshold at 10 TTC/100ml has potentially important implications for public health policy. The current standard set by WHO for TTC in drinking

water is no detectable levels in 100 ml. This standard is an acceptable requirement for most settings. However, in some situations where water or treatment methods are scarce or unavailable, this study shows that it may be acceptable to set the standard at <10 TTC/100ml. One notable example is in emergency and humanitarian settings. The Sphere Project which sets minimum standards for "key life-saving sectors" for humanitarian settings, currently sets the minimum standard for water quality of "no fecal coliforms per 100ml at the point of delivery." (The Sphere Project, 2011). However, when the first guidelines were published, the standard was "no more than 10 fecal coliforms per 100ml at the point of delivery for undisinfected supplies." (The Sphere Project, 2000). The results presented here provide support for a potential return to that standard.

Given the conflicting evidence from previous studies and the current study, more research on this topic should be done. Pooling data from multiple studies is a useful method to avoid limitations due to study size that were present in many of the previous studies, particularly if the studies used comparable methods to measure water quality and cases of diarrhea. However, we also recommend that this issue be addressed as the primary objective of prospective studies. Designing a study with statistical power to specifically address this issue will overcome the main limitation of previous studies and studying the effect prospectively will avoid some of the other methodological limitations found here. For example, a study that measured water quality followed by diarrhea status three to seven days afterward rather than measuring water quality and asking about past diarrhea may provide a better estimate of what level of exposure leads to diarrhea. Should enough prospective studies be conducted, another analysis such as this one will

provide a more thorough evaluation of the association between TTC in drinking water and diarrhea.

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Table 1: Description of Studies From Which Data was Obtained and Analyzed

Lead Author	Year	Country	Study Design	Cited Water Quality Measurement Method	Case Definition	Recall Period for Self-reported Diarrhea
Clasen	2005	Colombia	RCT	APHA	3+ Loose stools / 24h	7 Days
Clasen	2006	Bolivia	RCT	APHA	3+ Loose stools / 24h	7 Days
Clasen	2014	India	RCT	APHA	WHO*	7 Days
Boisson	2009	Ethiopia	RCT	Not specified[§]	Local term "tekmat"	7 Days
Boisson	2010	DRC	RCT	APHA	3+ Loose stools / 24h	7 Days
Peletz	2011	Zambia	Cross-sectional	Not specified[§]	WHO*	7 Days
Peletz	2012	Zambia	RCT	APHA	WHO*	7 Days

* The WHO definition of diarrhea is three or more loose or watery stools in a 24 hour period (World Health Organization, 2005)

[§]Though no specific method was cited, the method described in these articles followed the APHA membrane filtration method

Table 2: Characteristics of individual studies included in aggregated data set

	Cross-Sectional		Randomized Controlled Trials					Combined	
	Zambia CS	Ethiopia	Colombia	Chiñiri	Zambia RCT	DR Congo	India	All Studies	All Exc. India
Population									
Total Households	254	314	137	59	120	231	2902	4017	1115
Total Individuals	1246	1516	681	317	615	1104	21039	26518	5479
Age									
Mean (SD)	15.61 (14.27)	21.82 (18.28)	19.02 (16.8)	20.77 (19.05)	14.76 (14.33)	21.59 (18.53)	26.8 (20.9)	26.47 (20.41)	19.25 (17.23)
Median	11	16	14	14	10	16	26	25	14
<5 (%)	374 (31.9)	196 (12.9)	142 (20.9)	60 (19.0)	193 (34.0)	185 (16.8)	4298 (20.8)	5448 (20.9)	1150 (21.5)
5-15 (%)	307 (26.1)	534 (35.2)	231 (34.0)	108 (34.2)	155 (27.3)	348 (31.6)	2723 (13.1)	4406 (16.9)	1683 (31.4)
>16 (%)	493 (42.0)	786 (51.8)	307 (45.1)	148 (46.8)	220 (38.7)	568 (51.6)	13691 (66.1)	16213 (62.2)	2522 (47.1)
Follow-up Rounds	-	-	3	2	12	14	10	14	14
Diarrhea									
Baseline Prevalence	13.19	8.53	21.77	-	11.89	11.84	-	12.53	12.53
Mean Prevalence for Follow-up (SD)	-	-	7.3 (3.23)	3.88 (0.13)	2.33 (1.52)	2.84 (1.46)	4.43 (2.35)	3.73 (2.31)	2.94 (1.82)
Total Observations	1246	1517	2736	634	7588	3970	179690	197381	17691
Observations with DD and WQ	1083	1159	1977	542	6131	3681	30479	45052	14573
Season									
Dry (%)	174 (14.0)	1470 (96.9)	597 (21.8)	634 (100)	3430 (50.4)	1970 (49.6)	25321 (14.1)	33596 (17.1)	8275 (48.9)
Rainy (%)	1072 (86.0)	47 (3.1)	2139 (78.2)	0 (0.0)	3375 (49.6)	2000 (50.4)	154369 (85.9)	163002 (82.9)	8633 (51.1)

Table 3: Total cases of diarrhea, total observations, and prevalence for each study by category

	Cases/Observations (Prevalence)							
	Colombia	Chiñiri	Zambia CS	Zambia RCT	Ethiopia	DR Congo	India	All Studies
Total	281/2441 (11.5%)	23/592 (3.9%)	163/1,236 (13.2%)	222/6,671 (3.3%)	128/1,500 (8.5%)	187/3,690 (5.1%)	6,800/156,357 (4.3%)	7,804/164,683 (4.5%)
Age Category								
<5	130/501 (25.9%)	16/114 (14.0%)	83/372 (22.3%)	167/2,045 (8.2%)	28/194 (14.4%)	87/624 (13.9%)	2,616/26,446 (9.9%)	3,127/30,296 (10.3%)
5-15	85/822 (10.3%)	4/205 (2.0%)	25/306 (8.2%)	19/1,721 (1.1%)	35/533 (6.6%)	29/1,214 (2.4%)	901/26,733 (3.4%)	1,098/31,534 (3.5%)
>15	64/1105 (5.8%)	3/271 (1.1%)	48/488 (9.8%)	32/2,396 (1.3%)	65/773 (8.4%)	71/1,843 (3.9%)	3,283/103,171 (3.2%)	3,566/110,047 (3.2%)
p-value*	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	<0.001	<0.001
TTC Category								
<1	41/590 (6.9%)	11/345 (3.2%)	44/341 (12.9%)	41/2,111 (1.9%)	11/219 (5.0%)	37/1,053 (3.5%)	384/9,749 (3.9%)	569/14,408 (3.9%)
1-10	24/342 (7.0%)	1/50 (2.0%)	8/97 (8.2%)	23/986 (2.3%)	9/69 (13.0%)	14/430 (3.3%)	70/1,901 (3.7%)	149/3,875 (3.8%)
11-100	74/602 (12.3%)	5/97 (5.2%)	14/148 (9.5%)	39/1,232 (3.2%)	24/370 (6.5%)	20/265 (7.5%)	211/5,466 (3.9%)	387/8,180 (4.7%)
101-1000	54/452 (11.9%)	4/51 (7.8%)	55/401 (13.7%)	68/1,314 (5.2%)	39/383 (10.2%)	76/1,394 (5.5%)	299/7,159 (4.2%)	595/11,154 (5.3%)
>1000	-	-	27/160 (16.9%)	50/994 (5.0%)	15/118 (12.7%)	40/548 (7.3%)	286/6,206 (4.6%)	418/8,026 (5.2%)
p-value*	0.002	0.321	0.206	<0.001	0.023	0.001	0.167	<0.001
Season								
Dry	74/398 (18.6%)	23/592 (3.9%)	16/174 (9.2%)	128/3,378 (3.8%)	128/1,453 (8.8%)	62/1,818 (3.4%)	776/22,023 (3.5%)	1,207/29,836 (4.0%)
Rainy	207/2043 (10.1%)	-	147/1,062 (13.8%)	94/3,293 (2.9%)	0/47 (0.0%)	125/1,872 (6.7%)	6,024/134,334 (4.5%)	6,597/142,651 (4.6%)
p-value*	<0.001	-	0.093	0.033	0.033	<0.001	<0.001	<0.001

Totals for each category do not always sum to the total cases due to missing data

* p-values for Chi-square test of homogeneity

Table 4: Water Quality Measurements by Study

		CFU TTC / 100 mL		Log10 TTC		Number of Households per TTC Category (%)				
Study	N	Mean (SD)	Median	Mean (SD)	Median	<1	1-10	11-100	101-1000	>1000
Colombia	401	77.81 (115.84)	17	1.15 (0.94)	1.23	104 (25.9)	71 (17.7)	127 (31.7)	99 (24.7)	-
Chiñiri	101	35.26 (87.66)	0	0.58 (0.88)	0	64 (63.4)	9 (8.9)	16 (15.8)	12 (11.9)	-
Zambia CS	234	700.26 (2130.42)	74	1.6 (1.3)	1.87	71 (30.3)	21 (9.0)	29 (12.4)	84 (35.9)	29 (12.4)
Zambia RCT	1313	668.7 (1802.87)	20	1.39 (1.3)	1.3	421 (32.1)	196 (14.9)	240 (18.3)	261 (19.9)	195 (14.9)
Ethiopia	234	451.37 (1792.05)	85	1.73 (1.06)	1.93	47 (20.1)	12 (5.1)	72 (30.8)	79 (33.8)	24 (10.3)
DR Congo	815	1548.45 (5721.82)	140	1.64 (1.38)	2.15	236 (29.0)	99 (12.1)	56 (6.9)	319 (39.1)	105 (12.9)
India	4902	686.81 (1147.59)	60	1.66 (1.32)	1.78	1528 (31.2)	300 (6.1)	874 (17.8)	1180 (24.1)	1020 (20.8)
Overall	8000	726.4 (2235.6)	47	1.57 (1.3)	1.67	2471 (30.9)	708 (8.8)	1414 (17.7)	2034 (25.4)	1373 (17.2)

Table 5: Adjusted Odds Ratios of Diarrhea for Log 10 TTC / 100 ml*

	All Ages		Children <5	
Study	Adj. OR (95% CI)	p-value	Adj. OR (95% CI)	p-value
Zambia CS	1.14 (0.93-1.42)	0.192	1.26 (0.97-1.63)	0.087
Ethiopia	1.42 (1.00-2.01)	0.049	1.10 (0.71-1.71)	0.657
Chiñiri†	1.62 (0.97-2.70)	0.063	1.56 (0.81-3.04)	0.186
Colombia	1.60 (1.24-2.07)	<0.001	1.66 (1.14-2.41)	0.008
Zambia RCT	1.44 (1.28-1.63)	<0.001	1.38 (1.20-1.57)	<0.001
DR Congo	1.21 (1.06-1.39)	0.006	1.30 (1.07-1.57)	0.007
India	1.04 (0.99-1.10)	0.099	1.12 (1.03-1.21)	0.010
Combined	1.12 (1.08-1.18)	<0.001	1.18 (1.11-1.26)	<0.001
Combined Except India	1.36 (1.26-1.47)	<0.001	1.33 (1.21-1.46)	<0.001

*All studies were adjusted for categorical ages (<5, 5-15, >15) and season (rainy/dry) except Chiñiri which was adjusted only for age because all observations occurred in the same season.

†The Chiñiri study is only adjusted for age categories as all measurements occurred during the dry season.

Table 6: Diarrhea Odds Ratios for Categories of Thermotolerant Coliforms in Household Drinking Water

	TTC / 100 mL Drinking Water[§]				Linear Trend Test	
	1 to 10	11 to 100	101 to 1000	>1000	Slope (95% CI)	p-value
Zambia CS						
All Ages	0.71 (0.24-2.09)	0.84 (0.34-2.08)	1.28 (0.68-2.44)	2.13 (0.87-5.21)	0.06 (-0.14-0.25)	0.580
Children <5	0.26 (0.05-1.47)	0.84 (0.28-2.48)	1.60 (0.74-3.46)	2.31 (0.75-7.08)	-0.11 (-0.34-0.12	0.346
Ethiopia						
All Ages	4.09 (0.81-20.65)	1.16 (0.38-3.54)	2.49 (0.87-7.10)	3.64 (0.96-13.79)	0.31 (-0.17-0.79)	0.200
Children <5	3.33 (0.45-24.48)	2.20 (0.49-9.68)	2.15 (0.52-8.85)	0.87 (0.08-9.26)	0.10 (-0.34-0.54)	0.650
Chiñiri						
All Ages	0.64 (0.08-5.46)	1.79 (0.56-5.74)	3.10 (0.80-11.99)	-	0.27 (-0.44-0.99)	0.456
Children <5	1.13 (0.11-11.97)	3.69 (0.79-17.19)	0.95 (0.09-10.06)	-	0.08 (-0.62-0.77)	0.830
Colombia						
All Ages	1.69 (0.83-3.46)	1.98 (1.12-3.52)*	3.88 (1.93-7.83)*	-	0.63 (0.19-1.08)	0.006
Children <5	1.15 (0.37-3.61)	2.34 (1.03-5.35)*	3.62 (1.32-9.89)*	-	0.60 (-0.05-1.26)	0.072
Zambia RCT						
All Ages	1.27 (0.74-2.18)	1.94 (1.20-3.15)*	3.18 (2.02-4.99)*	3.33 (2.05-5.41)*	0.55 (0.31-0.79)	<0.001
Children <5	1.29 (0.71-2.37)	1.81 (1.05-3.10)*	2.85 (1.72-4.71)*	2.90 (1.68-4.99)*	0.48 (0.23-0.72)	<0.001
DR Congo						
All Ages	1.12 (0.55-2.30)	2.00 (1.01-3.96)*	1.51 (0.90-2.54)	2.26 (1.28-4.00)*	0.24 (0.05-0.43)	0.012
Children <5	1.17 (0.43-3.17)	2.28 (0.83-6.24)	1.99 (0.99-4.00)	2.42 (1.08-5.46)*	0.35 (0.04-0.66)	0.027
India Sanitation						
All Ages	1.06 (0.78-1.44)	1.08 (0.88-1.32)	1.08 (0.90-1.30)	1.20 (0.99-1.45)	0.04 (0.00-0.08)	0.045
Children <5	1.10 (0.65-1.86)	1.48 (1.06-2.07)*	1.11 (0.82-1.51)	1.62 (1.19-2.21)*	0.11 (0.03-0.19)	0.009
Combined						
All Ages	1.02 (0.82-1.26)	1.21 (1.03-1.42)*	1.35 (1.16-1.57)*	1.49 (1.27-1.76)*	0.11 (0.07-0.16)	<0.001
Children <5	0.94 (0.68-1.31)	1.52 (1.20-1.93)*	1.50 (1.20-1.86)*	1.77 (1.40-2.24)*	0.18 (0.11-0.25)	<0.001
Combined Except India						
All Ages	1.21 (0.88-1.68)	1.66 (1.25-2.19)*	2.30 (1.76-2.99)*	2.97 (2.17-4.08)*	0.40 (0.28-0.52)	<0.001
Children <5	1.02 (0.67-1.56)	1.81 (1.29-2.55)*	2.28 (1.66-3.13)*	2.38 (1.62-3.51)*	0.35 (0.21-0.49)	<0.001

[§] <1 TTC/100ml was used as the reference group for calculating odds ratios

* Significant at an alpha of 0.05

Figure 1: Forest plot of adjusted odds ratios from multi-level logistic regression model with log 10 TTC as a continuous predictor. Models are adjusted for age as a categorical variable (<5, 5-15, >15) and season. The summary measures are also adjusted for study location. Points of effect measure are proportional to the number of observations.

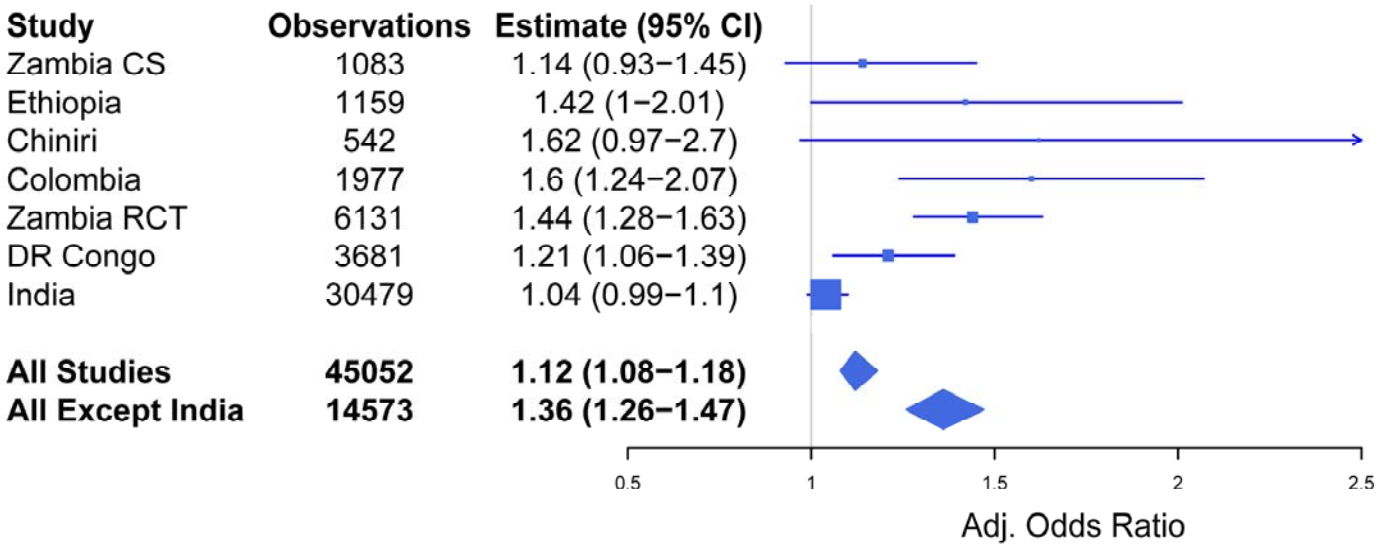

Figure 2: Adjusted odds ratios for WHO risk categories (with <1 TTC/100ml as references) for all studies combined and each study individually. Odds ratios are shown for all ages and for children under five separately.