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Health risks from exposure to untreated wastewater used for irrigation in the Mezquital Valley, Mexico: A 25-year update

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ABSTRACT

Wastewater reuse for agriculture is common worldwide; wastewater treatment, however, is rare in many countries, leading to high potential for exposure to harmful pathogens. Mexico City, one of the largest producers of untreated wastewater for agricultural use worldwide, was the site of key epidemiologic studies conducted in the 1990s. We both reviewed the literature on and conducted a cross-sectional study of diarrheal risk and wastewater contamination to provide an updated assessment of health risks and to inform an upcoming update of the 2006 WHO guidelines on wastewater reuse. We surveyed communities in the Mezquital Valley that use wastewater for irrigation and communities that use well water to compare the prevalence of self-reported diarrheal disease in children under five years old. Wastewater, well water, household environmental samples, and stool samples were collected and analyzed. Communities exposed to wastewater had a higher one-week prevalence of diarrhea (10%) compared to unexposed communities (5%). This association remained in an adjusted modified Poisson regression model (PR = 2.31, 95% CI 1.00, 5.31), but not when limited to households engaged in agriculture. Water quality indicators document differences between irrigation water from the two community groups. These results are in agreement with 25 population studies identified by our review that were conducted since or not included in the 2006 WHO guidelines and show consistent negative impacts of wastewater exposure on health. While overall diarrheal prevalence has declined when compared to studies conducted over 25 years ago in the same region, the association of diarrheal disease and wastewater exposure has remained and possibly increased. With rising urbanization worldwide, attention to these risks and wastewater treatment is becoming increasingly important.

1. Introduction

Reuse of wastewater and human excreta in agriculture is a long-standing practice worldwide and allows communities to cheaply fertilize crops, recycle nutrients, save clean water otherwise used

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for irrigation, and prevent contaminating bodies of water through wastewater dumping. However, constituents of wastewater such as potentially harmful pathogens, antibiotic resistant bacteria, and toxic or biologically disruptive chemicals can adversely affect human health if not properly controlled. Despite these health issues, treatment of wastewater is rare in many countries (Sato et al., 2013). Of particular importance are diarrheal diseases, which remain the second leading cause of death in children under five worldwide and result in about 800,000 child deaths each year (Liu et al., 2012). The relationship between diarrheal disease, as well as repeated subclinical infection, and nutritional stunting also poses long-term risks for children exposed to enteric pathogens (Checkley et al., 2008). We examined diarrheal risk in farming communities receiving untreated wastewater from Mexico City for agricultural irrigation.

Mexico City, one of the largest cities in the world, sends almost all its untreated wastewater through a series of uncovered canals to the Mezquital Valley, an agricultural area in the neighboring state of Hidalgo. It is the single largest such system in the world and has been operating for over 100 years (Siemens et al., 2008). In approximately 24 hours, the wastewater reaches the Valley, where a series of smaller canals move the wastewater into crop fields near individual communities. Agricultural workers use these canals to irrigate crops by flooding the fields with wastewater. Some of the water is stored in a large reservoir before distribution, while the remainder is sent directly to crop fields. Although existing laws aim to prevent the use of wastewater for irrigation of crops consumed by humans, there is potential for high levels of occupational exposure to untreated wastewater through direct contact with irrigated fields. The families and communities of field workers also are potentially exposed through tracking of pathogens by field workers on their clothes or shoes, through contact with crops grown for animal consumption, by small-scale production of crops consumed locally by humans, or by aerosolization of pathogens from the wastewater canals (Dickin et al., 2016; Paez-Rubio et al., 2005).

Currently there is a movement to encourage treating wastewater before reuse or returning it to the environment, including a specific Sustainable Development Goal (SDG) target to halve the proportion of untreated wastewater globally and to increase safe reuse (United Nations General Assembly, 2015). Treatment reduces the concentration of microbial pathogens in wastewater, thus reducing human exposure, and in theory leading to a decrease in the risk of adverse health outcomes. However, there is little epidemiologic evidence demonstrating the specific health benefits of wastewater treatment.

To this end, a treatment plant is near completion that will treat part of the wastewater from Mexico City before it reaches farms in the Mezquital Valley. The Atotonilco wastewater treatment plant will provide treated wastewater to some communities in the Mezquital Valley, but others will continue receiving untreated wastewater after the plant begins operation. This new plant, therefore, provides a unique opportunity to directly measure the impact of wastewater treatment on disease rates by comparing the health risks associated with varying levels of exposure to treated and untreated wastewater used in agriculture.

To provide a comprehensive picture of the risks of wastewater reuse, we conducted a literature review to summarize recent epidemiologic evidence. Unlike other recent reviews conducted on wastewater reuse (Dickin et al., 2016), we explicitly describe the associated health effects that have been measured and the shortfalls that remain in our understanding of wastewater and health. In this paper, we first describe the studies previously conducted in the Mezquital Valley that have been instrumental in developing wastewater reuse policy and then present a review of more recent epidemiologic evidence. To add to this literature, we conducted a cross-sectional study as an update to previous studies of the 1990s in the Mezquital Valley and to provide contemporary health risks associated with wastewater reuse prior to the opening of the Atotonilco treatment plant.

2. Methods

2.1. Review of epidemiologic studies on wastewater reuse

We reviewed the existing literature on wastewater reuse and health in three groups: 1) earlier studies conducted in the Mezquital Valley that were central sources for the 2006 World Health Organization (WHO) Guidelines for the Safe Use of Wastewater in Agriculture and Aquaculture (World Health Organization, 2006), 2) additional population studies included in the WHO guidelines, and 3) population studies that were not included in the WHO guidelines or that were conducted after the guidelines were created.

Recent studies were identified in PubMed using multiple combinations of search terms for wastewater (e.g. wastewater, waste water, grey water, sewage), reuse (e.g. reused, recycled, reclaimed, recovered), agricultural irrigation (e.g. agriculture, irrigation, crops, farm), and health outcomes (e.g. disease, diarrhea, pathogen, health, enteric, infection). Titles and abstracts of all search results were checked for relevance, and studies that measured health outcomes in a population were included. Studies that estimated outcomes in other ways, such as quantitative microbial risk assessments (QMRAs), were excluded. References cited in papers identified by this search were inspected for additional studies. In addition, studies that cited these references or those included in the WHO guidelines were identified using PubMed and Web of Science. Finally, a recent systematic review that focused on routes of exposure to wastewater was evaluated for additional studies not captured by our search (Dickin et al., 2016).

2.2. Cross-sectional study in the Mezquital Valley

We conducted a cross-sectional study in the Mezquital Valley as an update to earlier studies in this area. We collected baseline sociodemographic measurements and measured health risks among communities that are exposed to untreated wastewater through agricultural use and unexposed communities that irrigate with well water. We compared the prevalence of diarrheal disease in children under five years old between the two community groups, as well as the types of bacterial, viral, and protozoan pathogens found in stool, household water and dust, and wastewater or well water samples.

2.3. Study population

Communities in the Mezquital Valley were selected for sampling from three groups based on their wastewater use. Group A includes communities near Tula, Hidalgo that currently receive untreated wastewater for irrigation. When the treatment plant opens, these communities are expected to begin receiving treated wastewater. Group B includes communities near Tetepango, Hidalgo that also currently receive untreated wastewater for irrigation. When the treatment plant opens, these communities will not be impacted and will continue receiving untreated wastewater. Group C comprises communities in Tecozautla, Hidalgo, which use well water for irrigation and do not receive wastewater. Because this study was performed before the treatment plant opening, Groups A and B are collectively considered exposed to untreated wastewater and Group C is considered unexposed. Community groups were progressively sampled over three sequential weeks in

May and June 2015, corresponding with the rainy season and the usual annual peak incidence of diarrheal disease in children under five in the state of Hidalgo. (Hidalgo Ministry of Health, 2014). Within these groups, individual communities that were known to have significant agricultural activity were preferentially selected and larger municipal seats were excluded due to limited agricultural activity, although work in agriculture was not a criterion for inclusion in the study. Eligible households were those with at least one child under five years old living in the household and with a parent or legal guardian of the child present. Using information from community leaders and members, neighborhoods with higher numbers of children under five were targeted. Referral sampling was sometimes used to find additional households with eligible children.

2.4. Household survey

At each participating household, a parent or legal guardian completed a survey with questions related to sociodemographics, agricultural activities, household characteristics, hygiene practices, information about children under five, and self-reported diarrhea of children, among others. Diarrheal disease was reported as a child under five years old having three or more loose stools in a day within the past seven days (World Health Organization, 2013). Trained interviewers also took anthropometric measurements of one child under five per household and documented observations of the household, such as sanitation and hygiene availability. Survey responses and other data were recorded on cell phones using the Qualtrics offline application (Qualtrics, 2015).

2.5. Stool samples

Each household that reported a child with diarrheal disease was asked to provide a stool sample from that child for analysis of pathogens. For each household with a case, a participating household in the same community without a diarrheal case was asked to provide a stool sample as a control. Controls were matched to cases by age within six months. Stool samples were collected by the parent or legal guardian the same night or at the first stool the following morning. Stool was taken from a diaper or plastic toilet with a sterile tongue depressor and was placed and sealed in Cary-Blair medium. The samples were picked up the following day and stored on ice until being shipped or driven to the laboratories at the Universidad Nacional Autónoma de México (UNAM) in Mexico City to be processed and analyzed. Preliminary analyses of stool samples tested for several pathogens, including *Escherichia coli*, *Salmonella* spp., *Campylobacter jejuni*, *Shigella* spp., *Aeromonas hydrophila*, *Cryptosporidium parvum*, *Giardia lamblia,* rotavirus, norovirus, and adenovirus. In addition, stool samples were processed for the detection of gram-negative bacteria producing extended spectrum beta-lactamase (ESBL), reduced susceptibility to most of the carbapenem agents (KPC), and detection of vancomycin resistant enterococci (VRE) in *Enterococcus faecalis* and *Enterococcus faecium*. For details on transport and laboratory methods for stool processing, see Supplemental Materials.

2.6. Environmental samples

Within Groups A, B, and C, equal subsets of diarrheal cases and controls that provided stool samples were randomly selected for collection of household water samples and dust samples. Household water was requested as the water used for domestic activities: preferably tap water from the municipal source. Dust samples were collected from both inside and outside each household. In addition to these household samples, community-level wastewater and well water samples were collected for pathogen testing. Wastewater samples were collected directly from the wastewater canals serving Groups A and B. Water samples from wells serving communities in Group C were collected from pumps made available by local well operators. Two wastewater canals were sampled near communities in Group A and two near communities in Group B. Samples were taken from three or four locations along each canal and pooled for analysis. Three wells were sampled among communities in Group C. The physicochemical properties of wastewater and well water were measured. Wastewater, well water, and household water were analyzed for fecal coliforms (FC), fecal enterococci (FE), *E. coli*, *C. parvum*, *G. lamblia*, VRE, KPC, and ESBL. Dust samples were tested for fecal indicators, *E. coli*, *Salmonella* spp., KPC, and ESBL. For details on processing and analytic methods for environmental samples, see Supplemental Materials.

2.7. Statistical analysis

Diarrheal prevalence was compared between exposed communities (Groups A and B) and unexposed communities (Group C) at the household level. If one or more children in a household had diarrhea in the previous week, household diarrhea = 1, otherwise household diarrhea = 0. Crude statistical analysis was conducted using a log-binomial model. Because adjusted log-binomial models did not converge, adjusted analyses were conducted with Poisson models with robust variance. These models were chosen for their ability to directly estimate prevalence ratios between exposure groups (Barros and Hirakata, 2003; Coutinho et al., 2008; Deddens and Petersen, 2008). Household diarrhea was used as the dependent variable and the primary independent variable was a binary measure of living in a community exposed or unexposed to wastewater. A macro provided by the WHO was used to convert anthropometric data into z-scores (World Health Organization, 2016). Stunting and wasting were defined as greater than two standard deviations below the means of length/height-for-age and weight-for-age, respectively.

Potential confounding variables were decided conceptually then added to the model individually to measure their effect on the association between wastewater exposure and diarrheal disease. Variables were excluded if there was little to no effect. To explore effect modification in households that are directly exposed to wastewater through agricultural work, an additional model was run using only households with at least one person who works in the fields. Data were analyzed using SAS® software Version 9.4 (SAS Institute Inc, 2012).

2.8. Comparing diarrheal prevalence

Using estimates of rates of underreporting for different diarrheal recall periods, we compared our results to previous studies conducted in this area. Arnold et al. (2013) found that diarrheal recall dropped to 16–29% of the true prevalence beyond one week of recall, while approximately 90% of cases were captured in the first week of recall. Using the more conservative approximation of the proportion of cases captured in the earlier of the two weeks, estimated two-week recall should measure 90% of true cases in the first week and about 30% of cases in the earlier week.

The estimated diarrheal prevalence using two-week recall is approximately equal to $0.9 + 0.3 = 1.2$ times the true weekly prevalence. Thus, estimated two-week prevalence $= 1.2 \times$ true one-week prevalence and estimated one-week prevalence $= 0.9 \times$ true one-week prevalence. Isolating the true one-week prevalence in each case shows the two estimates are related by the formula

$$\frac{estimated\ one-week\ prevalence}{0.9} = \frac{estimated\ two-week\ prevalence}{1.2}$$

Estimated one-week prevalence is then equal to the estimated two-week prevalence times $\frac{0.9}{1.2}$ or 0.75. We multiplied two-week prevalence from two earlier Mezquital studies (Blumenthal et al., 2001; Cifuentes, 1998) by 0.75 to create estimates of the prevalence these studies would have measured using one-week recall.

2.9. Human subjects approval

Study protocols were approved by institutional review boards at the University of Michigan (HUM00090424), Universidad Nacional Autónoma de México Facultad de Medicina (CIE-FM 153/2014), Instituto Nacional de Salud Pública (CI/057/2016), and the Hidalgo State Secretaría de Salud. Parents or legal guardians gave written, informed consent prior to participation or collection of data.

3. Results

3.1. Review of epidemiologic studies on wastewater reuse

Due to its size and long history, the Mexico City wastewater reuse system was used as the study site for the key epidemiological-microbiological studies in the 1990s that informed the 2006 WHO Guidelines for the Safe Use of Wastewater in Agriculture and Aquaculture (Blumenthal and Peasey, 2002; Blumenthal et al., 2000; Siebe and Cifuentes, 1995; World Health Organization, 2006). Blumenthal and Cifuentes led a series of studies in the Mezquital Valley on wastewater and health (Table 1). Three cross-sectional surveys were conducted between 1990 and 1992 during rainy and dry seasons. These studies compared between 556 and 850 households using untreated wastewater for irrigation and 470–930 households using rainwater for irrigation on two-week self-reported diarrheal prevalence and infection with *Ascaris lumbricoides*, *G. lamblia*, and *Entamoeba histolytica*. In the dry season, Blumenthal et al. (2001) found that use of untreated wastewater was associated with higher prevalence of *A. lumbricoides* infection in children under five (OR = 18.01, 95% CI 4.10, 79.16) and in persons older than five (OR = 13.49, 95% CI 6.35, 28.63), and higher two-week prevalence of diarrheal disease in children under five (19% *vs.* 14%, OR = 1.75, 95% CI 1.10, 2.78). During the rainy season, Cifuentes (1998) found untreated wastewater use to be associated with higher two-week prevalence of diarrheal disease in children under five (29% *vs.* 23%, OR = 1.33, 95% CI 0.96, 2.18), and higher prevalence of *A. lumbricoides* infection in children under five (OR = 5.71, 95% CI 2.44, 13.36) and persons older than five (OR = 13.18, 95% CI 7.51, 23.12). Cifuentes et al. (1994, 2000) also found higher risks of *E. histolytica* infection for children aged five through fourteen (RR = 1.40, 95% CI 1.07, 1.72), and no association between wastewater reuse and infection with *G. lamblia* (OR = 1.07, 95% CI 0.84, 1.36) in the rainy season.

In their 2006 guidelines, the WHO reported evidence from 21 population studies in addition to those conducted in Mexico (Table 1). Only two of these additional studies included diarrheal disease as an outcome, while seven studies focused on infections by *A. lumbricoides*, *Trichuris trichiura*, or hookworm and the rest studied various infections or illnesses. In general, these studies found higher risks of infection or illness associated with wastewater reuse. For example, Fattal et al. (1986) found higher risk of enteric diseases among children under five in Israel after their villages switched from non-wastewater irrigation to wastewater irrigation (IRR = 1.91, 95% CI 1.30, 2.80).

Most of these studies that form the basis of the WHO guidelines were carried out over twenty years ago, including those from Mexico. Our review of more recent literature found 25 additional population studies that assessed the health risks associated with wastewater exposure and were not included in the WHO guidelines or were published after their release (Table 2) (Agunwamba, 2001; Amahmid and Bouhoum, 2005; Anh et al., 2007, 2009; Ceylan et al., 2003; Devaux et al., 2001; El Kettani et al., 2008; Ensink et al., 2005, 2006, 2008; Feenstra et al., 2000; Fuhrimann et al., 2014; Gumbo et al., 2010; Hien et al., 2007; Lekouch et al., 1999; Melloul et al., 2002; Pham-Duc et al., 2011, 2013, 2014; Srikanth and Naik, 2004; Trang et al., 2006, 2007a, 2007b, 2007c, 2007d). Beyond these population studies, many other studies have been conducted that focused on wastewater quality and microbial risk assessments without measuring health in a population (Al-Hammad et al., 2014; Amha et al., 2015; Antwi-Agyei et al., 2015; Chavez et al., 2011; Diallo et al., 2008; Downs et al., 1999; Ferrer et al., 2012; Fonseca-Salazar et al., 2016; Jiménez and Chavez, 2004; Lesser-Carrillo et al., 2011; Mazari-Hiriart et al., 2008; Navarro and Jiménez, 2011; Siemens et al., 2008). Nineteen of the 25 population studies focused on diarrheal diseases or enteric infections. Most studies found an increased risk of disease or infection associated with wastewater reuse, such as a two-fold increase in diarrheal prevalence among exposed farmers and their children compared to unexposed farmers and children (OR = 2.00, 95% CI 1.04, 3.85) in Pakistan (Feenstra et al., 2000).

Pham-Duc et al. (2014) conducted a nested case-control study within a Vietnamese cohort to determine risk factors for diarrheal disease among adults. While direct contact with wastewater was associated with increased risk of diarrhea (OR = 2.4, 95% CI 1.2, 4.7), participation in wastewater irrigation was not independently associated with diarrhea (OR = 1.0, 95% CI 0.4, 2.5). Two additional cross-sectional studies conducted by Pham-Duc et al. (2011, 2013) also found that wastewater irrigation was not associated with infection by helminths or *E. histolytica*. Two cross-sectional studies conducted by Ensink et al. (2005, 2006) in Pakistan found that communities not engaged in wastewater reuse had significantly lower prevalence of diarrhea, *Giardia* infections, and helminth infections compared to communities practicing wastewater irrigation; however, farmers engaged in wastewater irrigation had similar risks when compared to non-farming community members in the same villages. For a more detailed description of these population studies and their results, see Table 2.

3.2. Household survey

A total of 314 households were interviewed during the three-week period, including 158 exposed households (Groups A and B) and 156 unexposed households (Group C). Among exposed households, 108 (68%) included at least one person who worked in the fields in agriculture, ranching, or herding (Table 3). There were 128 unexposed households (82%) with at least one field worker. The average age of children under five years old was 31 months among exposed households and 30 months among unexposed households. Participating parents or guardians in both groups had an average of nine years of education. There were no differences of stunting or wasting between exposure groups, although there was a high overall prevalence of stunting (19%).

3.3. Prevalence of diarrheal disease

There were 24 households with at least one diarrheal case (8%). Sixteen of these households were among exposed households (10%)

Table 1
Summary of population studies assessing health risks associated with wastewater reuse that were described in the 2006 WHO Guidelines for the Safe Use of Wastewater in Agriculture and Aquaculture (Section 3.2).

Author, Year	Location	Study Design	Target Population	Outcome Variable(s)	Results	Effect Measure (95% CI[a])
Baumhogger, 1949; Krey, 1949	Darmstadt, Germany	NA[b]	General population of all ages in a city using untreated wastewater for irrigation compared to cities not reusing wastewater	*Ascaris* infections	Higher prevalence of *Ascaris* infection among exposed (50%) compared to unexposed (6%)	Prevalence Ratio (PR)[c] = 8.3
Blumenthal et al., 2001	Mezquital Valley, Hidalgo, Mexico	Cross-sectional survey conducted and stool samples collected during the dry season (n households = 850 exposed, 950 exposed to partially treated, 930 unexposed)	Agricultural workers and their family members living in three exposure groups: irrigating with untreated wastewater, irrigating with wastewater treated through dam stabilization, irrigating with rainfall	Diarrheal disease (two-week recall); *Ascaris* infection	Higher prevalence of diarrheal disease among those exposed to untreated wastewater compared to those unexposed for children <5 (19% *vs.* 14%) and for those 5+ (7% *vs.* 6%); higher prevalence of *Ascaris* infection among exposed compared to unexposed for children <5 (10% *vs.* 1%) and for those 5+ (7% *vs.* <1%)	Exposed to untreated wastewater *vs.* unexposed Diarrhea (0–4): Odds Ratio (OR)[d] = 1.75 (1.10,2.78) Diarrhea (5+): OR[d] = 1.34 (1.00,1.78) *Ascaris* (o-4): OR[d] = 18.01 (4.10,79.16) *Ascaris* (5+) OR[d] = 13.49 (6.35,28.63)
Bouhoum and Schwartzbrod, 1998	Marrakesh, Morocco	Cross-sectional collection of stool samples (n = 253 exposed, 275 unexposed)	Children living in a community reusing untreated wastewater for irrigation compared to children from an unexposed community	Helminth infections (including *Ascaris* and *Trichuris*)	Higher prevalence among exposed of any helminth infection (73% exposed *vs.* 30% unexposed), *Ascaris* infection (33% *vs.* 2%), and *Trichuris* infection (17% *vs.* 2%)	Any helminth: PR[c] = 2.43 *Ascaris*: PR[c] = 16.5 *Trichuris*: PR[c] = 8.5
Camann et al., 1986	Lubbock, Texas, United States	Cohort study including weekly illness reporting by phone call, semiannual blood samples, and stool samples collected before, during, and after major irrigation periods	General population of all ages living in community surrounding land treated with wastewater of varying quality through sprinkler irrigation	Enteric viruses	Higher seroprevalence during irrigation compared to baseline (5% *vs.* 3%)	OR = 2.10 (1.56,2.03)
Camann and Moore, 1987	Lubbock, Texas, United States	Cohort study including weekly illness reporting by phone call, semiannual blood samples, and stool samples collected before, during, and after major irrigation periods	General population of all ages living in community surrounding land treated with treated wastewater through sprinkler irrigation	Clinical viral infections	Higher prevalence of infection during irrigation for highest aerosol exposure group (8% low, 8% medium, 24% high, p = 0.06)	Highest exposure *vs.* lowest: PR[c] = 3.0
Cifuentes et al., 1994	Mezquital Valley, Hidalgo, Mexico	Cross-sectional survey conducted and stool samples collected during the rainy season (n households = 680 exposed, 520 exposed to partially treated, 700 unexposed)	Agricultural workers and their family members living in three exposure groups: irrigating with untreated wastewater, irrigating with wastewater treated through dam stabilization, irrigating with rainfall	Diarrheal disease (two-week recall); *Ascaris*, *Giardia*, and *E. histolytica* infections	No differences between exposed and unexposed for *Giardia* or *E. histolytica* infections; higher prevalence of diarrheal disease (30% *vs.* 23%) and *Ascaris* infection (15% *vs.* 3%) for exposed children <5 compared to unexposed children	Exposed to untreated wastewater *vs.* unexposed Diarrhea (0–5): Relative Risk (RR) = 1.3 (1.03,1.64) Diarrhea (5–14): RR = 1.7 (1.25,2.37) *Ascaris* (0–5): RR = 5.6 (2.92,10.83) *Ascaris* (5–14): RR = 15 (8,30) *Ascaris* (15+): RR = 11 (5.2,24)
Cifuentes, 1998	Mezquital Valley, Hidalgo, Mexico	Cross-sectional survey conducted and stool samples collected during the rainy season (n households = 848 exposed, 544 exposed to partially treated, 928 unexposed)	Agricultural workers and their family members living in three exposure groups: irrigating with untreated wastewater, irrigating with wastewater treated through dam stabilization, irrigating with rainfall	Diarrheal disease (two-week recall); *Ascaris* infection	Higher prevalence of diarrheal disease among those exposed to untreated wastewater compared to unexposed for children <5 (29% *vs.* 23%) but not for those 5+; higher prevalence of *Ascaris* infection for children <5 (14% *vs.* 3%) and those 5+ (9% *vs.* 1%)	Exposed to untreated wastewater *vs.* unexposed Diarrhea (0–5): OR[d] = 1.33 (0.96,1.85) Diarrhea (5+): OR[d] = 1.10 (0.88,1.38) *Ascaris* (0–5): OR[d] = 5.71 (2.44,13.36) *Ascaris* (5+): OR[d] = 13.18 (7.51,23.12)
Cifuentes et al., 2000	Mezquital Valley,	Cross-sectional survey conducted and stool samples collected during	Agricultural workers and their family members living in three exposure groups:	*Giardia* infections	Those exposed to partially treated wastewater had the	Untreated Wastewater *vs.* Unexposed: OR[d] = 1.07 (0.84,1.36)

Table 1 (*continued*)

Author, Year	Location	Study Design	Target Population	Outcome Variable(s)	Results	Effect Measure (95% CI[a])
	Hidalgo, Mexico	the rainy season (n households = 848 exposed, 544 exposed to partially treated, 928 unexposed)	irrigating with untreated wastewater, irrigating with wastewater treated through dam stabilization, irrigating with rainfall		highest prevalence of *Giardia* infection (11%) followed by untreated wastewater and unexposed (both 8%); children aged 1–4 had the highest prevalence in each group	Partially Treated Wastewater *vs.* Unexposed OR[d] = 1.22 (0.94,1.58)
Fattal et al., 1985	Israel (30 kibbutzim)	Blood samples collected (n = 69 exposed, 245 unexposed field workers, 498 unexposed non-field workers)	Adults aged 18+ in three exposure groups: workers exposed to treated wastewater including irrigation workers, plumbers, and plant operators; irrigation and pond workers not exposed to wastewater; unexposed workers in unrelated jobs including clerks and child care	Seroprevalence of antibodies for *Legionella*	No significant differences of seroprevalence for any *Legionella* species between groups (9% exposed field workers, 10% unexposed field workers, 6% unexposed non-field workers); lower seroprevalence for *L. pneumophila* serogroups 1–8 for unexposed non-field workers (4%, 4%, and 1%)	Exposed field workers *vs.* unexposed non-field workers: Serogroups 1-8 PR[c] = 3.14 (0.89,11.85)
Fattal et al., 1986	Israel (11 kibbutzim)	Natural experiment using health records (population n = 3040) comparing communities before and after switching their irrigation water source	General population of all ages in communities that switched from non-wastewater to sprinkler irrigation with partially treated wastewater or vice versa	Enteric diseases (typhoid fever, paratyphoid, salmonellosis, shigellosis, gastroenteritis including diarrhea, aseptic meningitis, infectious hepatitis, fever, coxsackie virus infections)	For children aged 0–4, wastewater use was associated with more enteric diseases when switching from non-wastewater to wastewater irrigation and when switching from wastewater to non-wastewater irrigation; no association was found for those aged 5–18 or 19+	Exposed to wastewater *vs.* unexposed Switching from unexposed to exposed Incidence Rate Ratio (IRR) = 1.91 (1.30,2.80) Switching from exposed to unexposed IRR = 2.03 (1.15,3.61)
Fattal et al., 1987	Israel (30 kibbutzim)	Blood samples collected (n = 228 exposed to aerosol wastewater, 227 exposed to non-aerosol wastewater, 310 unexposed)	General population of all ages in communities in three exposure groups: exposed to aerosol wastewater, exposed to wastewater but not aerosols, unexposed to wastewater	Seroprevalence for echovirus, coxsackievirus, and hepatitis A virus	Only echovirus type 4 seroprevalence was significantly different between exposure groups (83% among aerosol exposed children 0–5, 27% among exposed to non-aerosol, and 33% among unexposed)	Exposed to aerosols *vs.* unexposed Echovirus 4 (0–5): PR[c] = 2.5 Echovirus 4 (6–17): PR[c] = 2.0 Echovirus 4 (25+): PR[c] = 3.2
Habbari et al., 2000	Beni-Mellal, Morocco	Cross-sectional survey and stool samples collected (n = 740 exposed, 603 unexposed)	Children attending primary school living in an area where untreated wastewater is used for irrigation compared to an unexposed area	Helminth infections (*Ascaris* and *Trichuris*)	Higher prevalence of *Ascaris* infection (21% *vs.* 4%) but not *Trichuris* infection (0.4% *vs.* 0.3%) among exposed children compared to unexposed children	*Ascaris* PR[c] = 5.25
Hopkins et al., 1993	Santiago, Chile	Cross-sectional survey and blood samples collected (n = 1815)	Children and adults aged <35 years old randomly selected where vegetables were known to be irrigated with untreated wastewater	*Helicobacter pylori* seropositivity	Consumption of raw vegetables was associated with increased prevalence of seropositivity	Consumers of raw vegetables *vs.* non-consumers OR[d] = 3.25 (1.94,5.71)
Katznelson et al., 1976	Israel (207 kibbutzim)	Incidence of enteric diseases were obtained from health records with laboratory confirmation of diagnoses of bacterial infections	General population of all ages in kibbutzim practicing spray irrigation with partially treated wastewater compared to kibbutzim not using wastewater	Enteric diseases (salmonellosis, shigellosis, typhoid fever, infectious hepatitis)	Shigellosis was the most common disease and incidence was higher among the exposed group compared to the unexposed group; incidence of salmonellosis, typhoid fever, and infectious hepatitis were also higher among the exposed group	Exposed kibbutzim *vs.* unexposed kibbutzim Shigellosis RR = 2.2 Salmonellosis RR = 3.7 Typhoid Fever RR = 4.3 Infectious Hepatitis RR = 2.0
Khalil, 1931	Tora, Egypt	NA[b]	Prison population eating vegetables irrigated with untreated wastewater compared to the village population not using wastewater	*Ascaris* infections	Prevalence of *Ascaris* infection was higher in the exposed prison population (70%) compared to the village population (10%)	PR[c] = 7.0

(*continued on next page*)

Table 1 (*continued*)

Author, Year	Location	Study Design	Target Population	Outcome Variable(s)	Results	Effect Measure (95% CI[a])
Krishnamoorthi, 1973	India	Cross-sectional (n = 898)	Adult farm workers using untreated wastewater compared to unexposed farm workers	Helminth infections (*Ascaris* and hookworm)	Higher prevalence of *Ascaris* infection (47% *vs.* 13%) and hookworm infection (70% *vs.* 33%) among exposed farmers compared to unexposed farmers	Exposed farmers *vs.* unexposed farmers *Ascaris* PR[c] = 3.62 Hookworm PR[c] = 2.12
Linnemann et al., 1984	Muskegon County, Ohio, United States	Prospective cohort study (n = 35 exposed, 41 unexposed) with monthly examinations, blood samples, throat swabs, and rectal swabs; five-month follow-up reflecting the growing season	Workers at a spray irrigation site using wastewater partially treated through stabilization ponds compared to unexposed road commission employees	Clinical illnesses (respiratory, gastrointestinal, both, and other); viral seropositivity (poliovirus, coxsackievirus, echovirus, and hepatitis A)	No differences between groups on clinical illnesses (0.54 illnesses per worker per month among exposed *vs.* 0.58 illnesses among road workers); no differences of seropositivity to any virus between groups	Exposed to spray irrigation *vs.* unexposed workers Clinical illnesses IRR[c] = 0.93
Margalith et al., 1990	Israel (30 kibbutzim)	Blood samples were collected from kibbutzim residents (n = 1800) and overseas volunteers (n = 304); two samples were collected from kibbutzim residents over two years	Kibbutzim residents aged three months to 60+ years old divided into three exposure groups: exposed to wastewater through spray irrigation, exposed to wastewater without aerosolization, and unexposed to wastewater; additional participants were overseas volunteers aged 18–34, primarily from Europe or North America, who were working at the kibbutzim	Seroprevalence for poliovirus types 1, 2, and 3	There was no difference between exposure groups on seroprevalence of any poliovirus type (type 2: 93% among exposed to spray irrigation, 88% exposed without spray irrigation, 87% unexposed in first year); lower seroprevalence against poliovirus type 3 among volunteers (75%) compared to age-matched kibbutzim residents (86%)	Exposed to spray irrigation *vs.* unexposed Poliovirus Type 2 PR[c] = 1.07 Kibbutzim members *vs.* Foreign volunteers Poliovirus Type 3 PR[c] = 1.15
Melloul and Hassani, 1999	Marrakesh, Morocco	Cross-sectional stool sample collection (n = 390 exposed, 350 unexposed)	Children aged 3–15 living in a community reusing untreated wastewater for irrigation compared to children from an unexposed community	*Salmonella* infections	Higher prevalence of *Salmonella* infection in exposed children (33%) compared to unexposed children (1%)	PR[c] = 33.0
Sehgal and Mahajan, 1991	India	Cross-sectional (n = 2372)	General population in villages using untreated wastewater for irrigation and villages using treated wastewater, both compared to an unexposed village	*Giardia* infections	No significant prevalence differences found between villages exposed to untreated wastewater (12%), treated wastewater (16%), and unexposed villages (12%)	Exposed to untreated wastewater *vs.* exposed to treated wastewater PR[c] = 0.75
Shuval et al., 1984	Jerusalem, Israel	Reviewed multiple natural experiments and routinely collected data, making comparisons across time on infection prevalence	General population under multiple natural settings including consumption of crops irrigated with wastewater and subsequent removal of wastewater-irrigated crops form the market	Cholera, *Ascaris* infections, and *Trichuris* infections	After removal of vegetables grown with untreated wastewater from the market, prevalence of *Ascaris* infection fell from 35% to 1% and *Trichuris* infection fell from 13% to 5%; a rise in infections was seen after reintroducing wastewater-irrigated crops; during a cholera outbreak, removal of wastewater-irrigated crops was followed by a decline in cholera cases and the end of the epidemic	After removing vegetables *vs.* before *Ascaris* PR[c] = 35.0 *Trichuris* PR[c] = 2.60
Shuval et al., 1989	Israel (20 kibbutzim)	Surveillance using clinic data (total population n = 10,231)	General population of all ages in communities in three exposure groups: exposed to aerosol wastewater, exposed to wastewater but not aerosols, unexposed to wastewater	Enteric diseases (salmonellosis, amebiasis, campylobacterosis, diarrhea, shigellosis, giardiasis, viral hepatitis, and gastroenteritis)	Diarrhea and gastroenteritis were the most common enteric disease reported; no differences were seen for rate of enteric disease between aerosol-exposed (18.5 episodes per person per 100 years), exposed without aerosolization (15.1), and unexposed (17.2) persons	Exposed to aerosols *vs.* unexposed IRR[c] = 1.08 Exposed to aerosols *vs.* exposed to non-aerosol wastewater IRR[c] = 1.23

Table 1 (*continued*)

Author, Year	Location	Study Design	Target Population	Outcome Variable(s)	Results	Effect Measure (95% CI[a])
Srivastava and Pendey, 1986	India	Cross-sectional study comparing personal protection measures on risk of infection (n = 220)	Farmers reusing untreated wastewater	Parasitic infection and hookworm	Farmers with poor personal hygiene had higher prevalence of parasitic infection (82%) compared to those with good hygiene (26%); farmers that were barefoot had higher prevalence of hookworm infection (25%) compared to those with shoes (8%)	Poor hygiene *vs.* good hygiene Parasitic infection PR[c] = 3.15 Barefoot *vs.* non-barefoot Hookworm PR[c] = 1.13
Ward et al., 1989	Lubbock, Texas, United States	Cohort study including weekly illness reporting by phone call, semiannual blood samples, and stool samples collected before, during, and after major irrigation periods (n = 368)	General population of all ages living in community surrounding land treated with wastewater of varying quality through sprinkler irrigation	Rotavirus infection	Average annual rate of rotavirus infection of 6.8 infections per 100 subjects; 33% of children and 13% of adults tested positive for rotavirus; no association was found between rotavirus infection and wastewater spray irrigation (5% among high exposed *vs.* 3% among least exposed)	High aerosol exposure *vs.* low aerosol exposure PR[c] = 1.67

[a] 95% Confidence Interval.
[b] Articles not found through the University of Michigan library.
[c] Ratios and confidence intervals were calculated here or in WHO guidelines using prevalence/incidence data provided.
[d] Effect measure from article was estimated using an adjusted model.

and eight were among the unexposed (5%). There was at least one field worker in 18 of the 24 (75%) diarrheal case households, compared to 219 of the 290 (76%) households with no diarrheal cases (Table 4). Total years of education of the survey respondent, average age of the children under five in months, and number of children under five in the household were included as covariates in our full model. Additional variables found not to be important confounders included age of the parent or legal guardian, hygiene practices, drinking water source, breastfeeding practices, rotavirus vaccination status, wealth, and occupation.

Crude analyses (Table 5, Model 1) show a higher prevalence of diarrheal disease for exposed households, although this relationship is not statistically significant (PR = 1.98, 95% CI 0.87, 4.48). However, we do find a significantly higher prevalence of diarrheal disease among exposed households compared to unexposed households (PR = 2.31, 95% CI 1.00, 5.31) after adjustment (Table 5, Model 2). Education and the average age of children were found to be protective factors against diarrheal disease. Sub-analyses limited to households with at least one field worker (Table 5, Model 3) showed a higher prevalence of diarrheal disease in exposed households, although this relationship is weaker than that seen for all households and is not statistically significant (PR = 1.81, 95% CI = 0.75, 4.38).

3.4. Stool samples

A total of 37 stool samples were collected from 18 diarrheal cases and 19 controls. Of 24 diarrheal cases, six (25%) were not able to provide a stool sample or were not home when samples were picked up. The average age of children providing a stool sample was 20 months among cases and 25 months among controls. One or more potential pathogens were found in 13 (35%) samples (Table S1). None of the samples contained detectable rotavirus, *C. parvum*, *Salmonella* spp., *V. cholerae*, or VRE. Adenovirus, norovirus, *C. jejuni*, *Shigella* spp., *G. lamblia*, and *A. hydrophila* were found in a small number of samples. In addition, we found 35 (95%) samples positive for ESBL and 21 (57%) samples positive for KPC. Given the small number of positive stool samples, inference by exposure status is not possible. For detailed results of stool sample analysis, see Supplemental Methods.

3.5. Environmental samples

In general, the wastewater canals serving groups A and B exhibited similar physicochemical properties (Table S2). Small differences between these wastewater canals likely reflect temporal variation between sampling periods. Notably, well water samples contained nitrate, suggesting possible leaching and infiltration from the agricultural fields into groundwater. The four canals sampled had varied concentrations of fecal indicators (FC and FE) and pathogens (*C. parvum* and *G. lamblia*), although each canal had substantially higher concentrations than well water samples (Table 6). While each well water sample contained detectable FC and FE, none contained *C. parvum* or *G. lamblia.*

Household water and dust samples were collected from 24 total households, including 11 households with a case and 13 control households. Eight households provided samples from each community group A, B, and C. Household water from each group tested positive for fecal coliforms, fecal enterococci, and *G. lamblia* (Table S3). Household water in Groups A and C also tested positive for *C. parvum*, and households in Group A tested positive for antibiotic resistant KPC and VRE. ESBL was not detected in any household water samples. In dust samples, households from all three groups tested positive for fecal coliforms and suspected *E. coli*, while no households demonstrated the presence of *Salmonella*, ESBL, or KPC (Table S4). No clear differences were demonstrated between households with a case and control households for either water or dust samples, although comparisons are limited by sample size. For more detailed results from household water and dust sample tests, see Supplemental Materials.

3.6. Comparing diarrheal prevalence

The 1998 study that measured diarrheal disease in the

Table 2
Summary of population studies assessing health risks associated with wastewater reuse that were not included in the original WHO guidelines for wastewater reuse or that were published after the release of the guidelines.

Author, Year	Location	Study Design	Target Population	Outcome Variable(s)	Results	Effect Measure (95% CI[a])
Studies focused on diarrheal disease or enteric pathogens as primary outcome(s)						
Agunwamba, 2001	Nsukka, Nigeria	Cross-sectional questionnaire (n = 66) in a community using wastewater from stabilization ponds for irrigation	Farmers using wastewater and community members not associated with wastewater	Self-reported diarrheal disease, typhoid fever, and malaria	Self-reported incidence of diarrhea higher for consumers of crops grown with wastewater (2.02 episodes since last year) compared to non-consumers (1.15), and higher for wastewater users (2.73) compared to non-users (0.97)	Consumers *vs.* non-consumers Incidence rate ratio (IRR)[b] = 1.75 Wastewater users *vs.* non-users IRR[b] = 2.74
Amahmid and Bouhoum, 2005	Marrakesh, Morocco	Cross-sectional questionnaire and stool sample collection in an area reusing urban wastewater for irrigation (n = 323) and an unexposed community using well water (n = 287)	Children aged 2–14 living near a spreading area of untreated wastewater for irrigation and unexposed children from a similar control population	Helminth infections (*Ascaris*, *Trichuris*)	Prevalence of *Ascaris* infection higher in exposed children (13.3%) compared to unexposed children (1.7%); prevalence of *Trichuris* higher in exposed (13.3%) compared to unexposed (3.8%)	*Ascaris* Prevalence Ratio (PR)[b] = 7.82 *Trichuris* PR[b] = 3.50
Ceylan et al., 2003	Diyarbakir, Turkey	Cross-sectional blood samples taken from farmers (n = 46) and controls (n = 45)	Adult farmers using untreated wastewater for irrigation and neighbor controls who did not farm	Hepatitis E seropositivity	Higher prevalence of seropositivity among exposed farmers (34.8%) compared to unexposed neighbors (4.4%)	Odds Ratio (OR) = 11.5 (2.3,78.2)
El Kettani et al., 2008	Settat, Morocco	Cross-sectional questionnaire and stool sample collection in a population reusing wastewater (n = 214) and an unexposed population (n = 119)	General population aged 3–60+ in area reusing untreated wastewater compared to unexposed community	Parasitosis (including *Entamoeba coli, Endolimax nana, Pseudolimax butschlii, Giardia, Chilomastix masnili, Ascaris,* and *Enterobius vermicularis*)	Higher prevalence of at least one parasite among exposed (66%) compared to unexposed (32%) and higher prevalence of polyparasitism among exposed (17%) compared to unexposed (3%)	Any parasite Relative Risk (RR) = 2.08 (1.57,2.75) Polyparasitism PR[b] = 5.67
Ensink et al., 2005	Faisalabad, Pakistan	Cross-sectional questionnaire and stool samples from families of exposed farmers (n = 486), of textile workers (n = 742), and of unexposed farmers (n = 476)	Adults and their children aged 2–12 in three groups: farmers irrigating with untreated wastewater, textile workers living in the communities that reuse wastewater, and farmers in another community not engaged in wastewater reuse	Helminth infections (*Ascaris*, *Trichuris*, hookworm)	Adults: Higher prevalence of nematode infection for exposed farmers and textile workers compared to unexposed farmers; higher prevalence of hookworm infection for exposed farmers compared to textile workers Children: Higher prevalence of nematode infection for children of exposed farmers and textile workers compared to unexposed farmers; higher prevalence of hookworm infection for children of exposed farmers and textile workers compared to unexposed farmers	Exposed farmer families *vs.* unexposed farmer families Nematode infection (adults) OR[c] = 31.4 (4.1243.0) Nematode infection (children) OR[c] = 5.7 (2.1,16.0) Hookworm (children) OR[c] = 9.3 (2.0,43.0) Exposed textile worker families *vs.* unexposed farmer families Nematode infection (adults) OR[c] = 9.7 (1.2,78.0) Nematode infection (children) OR[c] = 4.1 (1.5,11.0) Hookworm (children) OR[c] = 6.9 (1.6,31.0)
Ensink et al., 2006	Faisalabad, Pakistan	Cross-sectional questionnaire and stool samples from families of exposed farmers (n = 486), of textile workers (n = 742), and of unexposed farmers (n = 476)	Adults and their children aged 2–12 in three groups: farmers irrigating with untreated wastewater, textile workers living in the communities that reuse wastewater, and farmers in another community not engaged in wastewater reuse	Diarrheal disease and *Giardia duodenalis* infections	Higher prevalence of *Giardia* infection for all participants in households of exposed farmers (77%) and textile workers (72%) compared to unexposed farmers (49%); higher prevalence of diarrhea in households of exposed farmers (5%) and textile workers (4%) compared to unexposed farmers (2%)	Exposed farmer families *vs.* unexposed farmer families *Giardia* OR[c] = 3.3 (2.5,4.4) Diarrheal disease PR[b] = 2.5 Exposed textile worker families *vs.* unexposed farmer families *Giardia* OR[c] = 2.4 (1.9,3.1) Diarrheal disease PR[b] = 2.0
Ensink et al., 2008	Hyderabad, India	Cross-sectional questionnaire and stool samples from families of farmers irrigating with	Farmers and their family members over two years old in three groups based on water source used for	Helminth infections (*Ascaris*, *Trichuris*, hookworm)	Higher prevalence of *Ascaris* infection in untreated wastewater group (10%) and partially treated group	Untreated *vs.* river water *Ascaris* OR = 5.3 (2.0,14.0)

Table 2 (*continued*)

Author, Year	Location	Study Design	Target Population	Outcome Variable(s)	Results	Effect Measure (95% CI[a])
		untreated wastewater (n = 240), partially treated wastewater (n = 354), and river water (n = 413)	irrigation: untreated wastewater, wastewater partially treated with a stabilization pond, and river water		(6.5%) compared to river water group (2.2%); higher prevalence of *Trichuris* infection among untreated group (8.3%) compared to partially treated (1.3%) and unexposed (1.6%); higher prevalence of hookworm infection among untreated group (55.4%) compared to partially treated (18.9%) and unexposed (24.2%)	*Trichuris* OR = 5.6 (1.8,18.0) Hookworm OR = 3.5 (2.2,5.5) Partially treated *vs.* river water *Ascaris* OR = 3.2 (1.2,8.6) *Trichuris* OR = 0.6 (0.2,2.5) Hookworm OR = 0.7 (0.4,1.1)
Feenstra et al., 2000	Punjab, Pakistan	Cross-sectional survey conducted (n = 204 exposed, 339 unexposed) and stool samples collected (n = 132 exposed, 152 unexposed)	Adult farmers that use untreated wastewater for irrigation and their children aged <12 compared to unexposed farmers and children from control villages	Self-reported diarrheal disease, skin and nail diseases, typhoid, cholera, and hepatitis; infection with intestinal parasites (*Giardia lamblia*, *Entamoeba coli*, *E. histolytica*, *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, *Taenia saginata*, and *Hymenolepis nana*)	Higher prevalence of diarrhea among exposed (11.7%) compared to unexposed (6.2%); only hookworm infection was significantly higher among exposed (38.6%) compared to unexposed (26.5%)	Diarrheal disease OR = 2.00 (1.04,3.85) Hookworm OR = 1.75 (1.06,2.89)
Fuhrimann et al., 2014	Kampala, Uganda	Cross-sectional survey and stool samples collected from adults in the five exposure groups (n = 915)	People living near a wastewater reuse system in one of five exposure groups: wastewater treatment plant workers, fecal sludge collectors, farmers, exposed community members at risk of flooding, and non-exposed community members	Parasite infection	Prevalence of infection was highest for farmers (75%) followed by exposed community members (53%), unexposed community members (45%), wastewater treatment plant workers (42%), and fecal sludge collectors (36%)	Exposed farmers *vs.* exposed community members PR[b] = 1.42 Exposed farmers *vs.* unexposed community members PR[b] = 1.67
Gumbo et al., 2010	Malamulele, South Africa	Cross-sectional survey (n = 194 exposed, 249 unexposed) and stool samples collected (n = 112 exposed, 131 unexposed)	Adult male farmers irrigating with treated wastewater and their children compared to control villages	Diarrheal disease, hookworm and *Giardia lamblia* infections	Higher prevalence of diarrhea among exposed participants compared to unexposed participants (12% *vs.* 6%); higher prevalence of hookworm infection (42% *vs.* 28%) and *Giardia* infection (39% *vs.* 36%) among exposed compared to unexposed	Diarrheal disease PR[b] = 2.0 Hookworm PR[b] = 1.50 *Giardia* PR[b] = 1.08
Hien et al., 2007	Hanoi, Vietnam	Nested case-control study for etiology of diarrheal cases from a cohort of randomly selected households (n = 400) monitored by weekly recall interviews over 18 months; stool samples collected from 111 case-control pairs	Pre-school children living in an area where untreated wastewater is used for agriculture	Diarrheal disease; diarrheal etiology (Rotavirus, *E. histolytica*, diarrheagenic *E. coli*, *Shigella*, *Salmonella*, *Campylobacter jejuni*)	Total diarrheal incidence rate of 0.63 episodes per child per year; samples from cases were more likely to contain any enteric pathogen than controls Pathogens associated with diarrhea were rotavirus and *E. histolytica*	Cases *vs.* Controls Contain any pathogen OR[c] = 3.55 (1.97,6.42) Rotavirus OR[c] = 4.5 (1.6,13.0) *E. histolytica* OR[c] = 4.4 (1.8,10.8)
Melloul et al., 2002	Marrakesh, Morocco	Cross-sectional stool samples collected to test for protozoa (n = 321 exposed, 287 unexposed) and *Salmonella* (n = 253 exposed, 350 unexposed)	Children aged 2–14 living near a spreading area of untreated wastewater for irrigation and unexposed children from a similar control population	Protozoa (*Giardia*, *E. histolytica*, *Entamoeba coli*, *Entamoeba hartmani*, *Endolimax nana*, *Iodamoeba butschlii*) and *Salmonella* infections	Exposed children had higher prevalence compared to unexposed children of protozoa infection (72% *vs.* 45%), *Giardia* infection (39% *vs.* 20%), amoeba infection (28% *vs.* 6%), and *Salmonella* infection (21% *vs.* 1%)	Protozoa PR[b] = 1.60 *Giardia* PR[b] = 1.95 Amoeba PR[b] = 4.67 *Salmonella* PR[b] = 21.0
Pham Duc et al., 2011	Hanam province, Vietnam	Case-control study (n = 46 cases, 138 controls) identified in a cross-sectional survey of households, with controls matched on sex, age group, and place of residence	General population of all ages living in communities where untreated wastewater is used for agriculture and aquaculture	Risk factors for *E. histolytica* infection	Socioeconomic status, contact with domestic animals, and hand washing were associated with *E. histolytica* infection; direct contact with wastewater-contaminated river water and use of wastewater-contaminated river water for irrigation were not associated with infection	Direct contact OR[c] = 0.4 (0.1,1.1) Irrigation OR[c] = 3.7 (0.4,33.1)

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Table 2 (*continued*)

Author, Year	Location	Study Design	Target Population	Outcome Variable(s)	Results	Effect Measure (95% CI[a])
Pham-Duc et al., 2013	Hanam province, Vietnam	Two cross-sectional surveys conducted and stool samples collected from randomly selected households without selecting households twice (n = 1425 individuals from 453 households)	General population of all ages living in communities where untreated wastewater is used for agriculture and aquaculture	Infections with *Ascaris*, *Trichuris*, hookworm, *E. histolytica*, *Entamoeba coli*, *Giardia lamblia*, *Cryptosporidium parvum*, and *Cyclospora cayetanensis*	Overall prevalence of infection by any helminth of 47%: largest prevalence for *Trichuris* (40%) and *Ascaris* (24%) Close contact to wastewater-contaminated river water was associated with higher prevalence of helminth infections; use of wastewater-contaminated river water for irrigation was not associated with helminth infections	Close contact (any helminth) OR[c] = 1.5 (1.1,2.2) Irrigation (any helminth) OR[c] = 1.1 (0.7,1.8)
Pham-Duc et al., 2014	Hanam province, Vietnam	Nested case-control study (n = 232 pairs) for etiology of diarrheal cases using a cohort (n = 867) following adults weekly for 12 months	Adults aged 16–65 living in communities where untreated wastewater is used for agriculture and aquaculture	Diarrheal disease; risk factors for diarrhea	Overall diarrheal incidence rate of 0.28 episodes per person per year Direct contact with wastewater-contaminated river water was associated with higher risk for diarrheal disease; other risk factors for diarrheal disease were contact with contaminated pond water, composting human excreta for <3 months, handling human or animal excreta in field work, lack of protective measures while working, handwashing, rainwater use for drinking, and eating raw vegetables the day before Use of wastewater-contaminated river water for irrigation was not associated with diarrhea	Direct contact OR[c] = 2.4 (1.2,4.7) Irrigation OR[c] = 1.0 (0.4,2.5)
Srikanth and Naik, 2004	Asmara, Eritrea	Cross-sectional stool sample collection (n = 75)	Farmers using untreated wastewater for irrigation	*Giardia* cysts	Found a high prevalence of *Giardia* cysts (45%)	NA
Trang et al., 2006	Nam Dinh city, Vietnam	Cross-sectional survey conducted (n = 570 exposed, 569 unexposed) and stool samples collected (n = 1088 total)	Adults aged 15+ living in households with someone engaged in agriculture in a community irrigating with untreated wastewater and an unexposed control community	*Ascaris*, *Trichuris*, and hookworm infections	Lower prevalence for exposed participants compared to unexposed of *Ascaris* infection and *Trichuris* infection; might reflect SES differences despite attempts to adjust for these factors	*Ascaris* OR[c] = 0.42 (0.32,0.54) *Trichuris* OR[c] = 0.45 (0.32,0.63)
Trang et al., 2007a	Hanoi, Vietnam	Nested case-control study (n = 163 pairs) for diarrheal etiology from a cohort study (n = 636) followed by weekly visits for 18 months	Adults aged 15–70 living in households with someone engaged in agriculture in a community reusing wastewater for agriculture and aquaculture	Diarrheal disease; diarrheal etiology; risk factors for diarrhea	Overall diarrheal incidence rate of 0.28 episodes per person per year Cases (29%) were more likely than controls (15%) to be identified as positive with an enteric pathogen; diarrheagenic *E. coli* (12%) was the most common pathogen, but none were statistically different between cases and controls Risk factors for diarrhea included direct contact with wastewater, handwashing, drinking water from well, consumption of raw foods, and contact with persons with diarrhea	Cases *vs.* controls Any pathogen OR[c] = 2.69 (1.55,4.65) Direct contact OR[c] = 1.98 (1.18,3.33)
Trang et al., 2007b	Hanoi, Vietnam	Cross-sectional survey conducted (n = 400 households) and stool samples collected (n = 620 adults, 187 children)	Adults aged 15–70 and preschool children aged <72 months living in households with someone engaged in agriculture in a community reusing	Helminth infections (*Ascaris*, *Trichuris*, and hookworm)	Overall prevalence of helminth infections of 39%: hookworm (22%), *Ascaris* (22%), *Trichuris* (10%) Risk factors for all three infections included being an adult, female gender, lack of	Direct contact (*Trichuris*) RR[c] = 2.14 (1.32,3.48)

Table 2 (*continued*)

Author, Year	Location	Study Design	Target Population	Outcome Variable(s)	Results	Effect Measure (95% CI[a])
			wastewater for agriculture and aquaculture		latrine, excreta composting for <1 month, and use of fresh excreta; year-round direct contact with wastewater was associated with *Trichuris* but not *Ascaris* or hookworm infections	
Studies focused on skin diseases as primary outcome						
Anh et al., 2007	Hanoi, Vietnam	Cross-sectional baseline examination with two follow-ups (n = 235 farmers) in communities engaged in wastewater reuse and an unexposed community using river, rain, and well water	Farmers engaged in aquatic plant culture in exposed and unexposed communities	Skin issues measured by dermatologist examination	Higher prevalence of dermatitis in exposed compared to unexposed farmers	OR[c] = 3.0 (1.1,7.7)
Anh et al., 2009	Phnom Penh, Cambodia	Cross-sectional baseline examination with two follow-ups (n = 650 adults) in communities reusing urban wastewater and unexposed communities using other sources	Adults aged 15+ living in communities reusing wastewater and unexposed communities	Skin issues measured by dermatologist examination	9.1% prevalence of dermatitis in exposed compared to 0 cases in non-exposed	NA
Trang et al., 2007c	Hanoi, Vietnam	Nested case-control study (n = 108 pairs) for risk factors for skin ailments from a cohort (n = 636) following adults by weekly visits for 12 months	Adults aged 15–70 living in households with someone engaged in agriculture in a community reusing wastewater for agriculture and aquaculture	Skin ailments	Overall incidence of skin ailments of 0.33 episodes per person per year Wastewater contact, female gender, fish farming job, and lack of protective measures taken were significant risk factors for skin ailments; among those with direct contact with wastewater, using wastewater exclusively for purpose other than irrigation was associated with more skin ailments	Direct contact OR[c] = 2.74 (1.29,5.82) Non-irrigation wastewater use OR[c] = 2.20 (1.03,4.69)
Trang et al., 2007d	Nam Dinh, Vietnam	Cohort study (n = 874) with monthly follow-up for 12 months	Adults aged 15+ living in households with someone engaged in agriculture in a community irrigating with untreated wastewater and an unexposed control community	Skin disease	Overall incidence of skin disease of 21% over one year; exposed participants had higher risk of skin disease compared to unexposed participants	RR[c] = 1.89 (1.39,2.57)
Studies focused on other primary outcomes						
Devaux et al., 2001	Clermont-Ferrand, France	Sentinel reporting of health issues by physicians/ pharmacists over three years after implementing wastewater system; repeated health questionnaires sent to field workers (n = 96 in first year); other farmers followed weekly for symptoms (n = 37) and matched to unexposed family members (n = 22)	Adult farmers and field workers engaged in spray irrigation with treated wastewater	Skin and digestive illnesses	Similar occurrence of nettle rashes, itchy skin, sunburns, and cuts over second and third years after the wastewater system was implemented; exposed farmers declared fewer symptoms than the unexposed group	NA
Lekouch et al., 1999	Marrakesh, Morocco	Cross-sectional collection of hair samples from children (n = 327)	Children aged 6–14 living near a spreading area of untreated wastewater for irrigation and unexposed children from a similar control population	Lead and cadmium levels	Mean lead level was higher among exposed children (14.8 μg/gram) compared to unexposed children (4.6); mean cadmium level was higher among exposed (4.6 μg/gram) compared to the unexposed (0.6)	Lead levels 3.22 times higher Cadmium levels 7.67 times higher

[a] 95% Confidence Interval.
[b] Ratios were calculated from prevalence/incidence data provided.
[c] Effect measure from article was estimated using an adjusted model.

Table 3
Characteristics of wastewater exposure groups (totals may vary due to missing values).

	Exposed to wastewater	Unexposed to wastewater	Total	p-value[a]
	n = 158	n = 156	n = 314	
Households with diarrheal case, No. (%)	16 (10.1)	8 (5.1)	24 (7.6)	.10[1]
At least one field worker in household, No. (%)	108 (68.4)	129 (82.7)	237 (75.5)	<.01[1]
Number of hours worked in the fields per week (highest in household), Mean (SD)	43.3 (24.6)	44.7 (16.16)	44.1 (20.3)	.64[2]
Years of education, Mean (SD)	8.84 (2.7)	8.9 (2.4)	8.9 (2.6)	.74[2]
Number of household assets (out of 13 possible)[b], Mean (SD)	5.4 (2.0)	5.5 (1.9)	5.4 (2.0)	.79[2]
Age of survey respondent in years, Mean (SD)	27.7 (8.1)	28.7 (9.3)	28.2 (8.7)	.42[2]
Age of children under five years old in months, Mean (SD)	31.0 (15.9)	29.7 (15.1)	30.3 (15.5)	.48[2]
Stunted (length/height-for-age), No. (%)	29 (19.1)	28 (18.5)	57 (18.8)	.91[1]
Wasted (weight-for-age), No. (%)	10 (6.7)	12 (8.1)	22 (7.4)	.65[1]
Number of people living in household, Mean (SD)	5.6 (2.2)	5.1 (1.7)	5.4 (2.0)	.07[3]
Number of positive hygiene observations made by interviewer (out of 10 possible binary variables)[c], Mean (SD)	7.6 (2.0)	7.8 (2.2)	7.7 (2.1)	.17[3]
Household breastfed at any point, No. (%)	145 (91.8)	141 (91.6)	286 (91.7)	.95[1]
Household breastfed at least six months, No. (%)	122 (87.8)	112 (83.0)	234 (85.4)	.26[1]
Use bottled water for primary drinking water source, No. (%)	146 (92.4)	41 (26.3)	187 (59.6)	<.0001[1]

[a] p-values compare exposed to unexposed and were obtained by 1: chi-square 2: independent *t*-test 3: Wilcoxon rank-sum test.
[b] Possible assets were electricity, television, refrigerator, landline telephone, cell phone, washing machine, microwave, computer, internet, flat-screen television, and up to three vehicles.
[c] Sum of the following observations: household free of trash, household free of feces, respondent's hands appeared clean, children's faces appeared clean, dishes appeared clean, area for food preparation seemed clean, yard free of feces, yard free of trash, children wore shoes outside, and no flies present in the kitchen or eating area.

Table 4
Characteristics of households with at least one diarrheal case and households without a diarrheal case (totals may vary due to missing values).

	Households with at least one case	Households without a diarrheal case	Total	p-value[a]
	n = 24	n = 290	n = 314	
Exposure Group	–	–	–	.22[1]
In Group A (Exposed), No. (%)	8 (33.3)	79 (27.2)	87 (27.7)	–
In Group B (Exposed), No. (%)	8 (33.3)	63 (21.7)	71 (22.6)	–
In Group C (Unexposed), No. (%)	8 (33.3)	148 (51.0)	156 (49.7)	–
Exposed to wastewater, No. (%)	16 (66.7)	142 (49.0)	158 (50.3)	.10[1]
At least one field worker in household, No. (%)	18 (75.0)	219 (75.5)	237 (75.5)	.95[1]
Number of hours worked in the fields per week (highest in household), Mean (SD)	43.8 (21.8)	44.1 (20.2)	44.1 (20.3)	.95[2]
Years of education, Mean (SD)	8.0 (3.5)	9.0 (2.5)	8.9 (2.6)	.19[2]
Number of household assets (out of 13 possible)[b], Mean (SD)	6.1 (3.0)	5.4 (1.9)	5.4 (2.0)	.24[2]
Age of survey respondent in years, Mean (SD)	26.0 (6.8)	28.4 (8.8)	28.2 (8.7)	.20[2]
Age of children under five years old in months, Mean (SD)	24.3 (13.5)	30.8 (15.5)	30.3 (15.5)	.05[2]
Number of people living in household, Mean (SD)	5.9 (2.0)	5.3 (2.0)	5.4 (2.0)	.07[3]
Number of positive hygiene observations made by interviewer (out of 10 possible binary variables)[c], Mean (SD)	7.9 (2.0)	7.7 (2.1)	7.7 (2.1)	.78[3]
Household breastfed at any point, No. (%)	23 (95.8)	263 (91.3)	286 (91.7)	.44[1]
Household breastfed at least six months, No. (%)	18 (85.7)	216 (85.4)	234 (85.4)	.97[1]
Use bottled water for primary drinking water source, No. (%)	16 (66.7)	171 (59.0)	187 (59.6)	.46[1]

[a] p-values compare cases to controls and were obtained by 1: chi-square 2: independent *t*-test 3: Wilcoxon rank-sum test.
[b] Possible assets were electricity, television, refrigerator, landline telephone, cell phone, washing machine, microwave, computer, internet, flat-screen television, and up to three vehicles.
[c] Sum of the following observations: household free of trash, household free of feces, respondent's hands appeared clean, children's faces appeared clean, dishes appeared clean, area for food preparation seemed clean, yard free of feces, yard free of trash, children wore shoes outside, and no flies present in the kitchen or eating area.

Table 5
Estimated associations between exposure to wastewater and under-five diarrheal prevalence among households in the Mezquital Valley.

	Model 1 n = 314	Model 2 n = 303	Model 3 n = 230
	Prevalence Ratio (95% CI[a])	Prevalence Ratio (95% CI)	Prevalence Ratio (95% CI)
Exposed to wastewater	1.98 (0.87, 4.48)	2.31 (1.00, 5.31)	1.81 (0.75, 4.38)
Total years of education of participant	–	0.88 (0.78, 1.00)	0.93 (0.79, 1.09)
Average age in months of children under five	–	0.97 (0.95, 1.00)	0.97 (0.94, 0.99)
Number of children under five in household	–	1.02 (0.47, 2.20)	1.32 (0.62, 2.82)

Model 1: Unadjusted log-binomial model.
Model 2: Multivariate Poisson model with robust variance among all households with non-missing covariates.
Model 3: Multivariate Poisson model with robust variance among households where at least one field worker resided.
[a] 95% CI = 95% Confidence Interval.

Table 6
Concentrations of select microbial organisms in wastewater and well water samples, with measurements from 1990s studies in the Mezquital Valley (Cifuentes et al., 1994, 1998, 2000).

	Wastewater Canals					Well Water		
	Canal 1[a] El Salto Tlamaco	Canal 2[a] Dendhó	Canal 3[b] El Alto Ajacuba	Canal 4[b] Tlamaco-Juandhó	Canals in 1990s Mezquital Studies	Well 1[c]	Well 2[c]	Well 3[c]
Fecal Coliform (CFU/100 mL)	1.28×10^7	1.38×10^7	1.21×10^7	7.73×10^6	10^8	0.7	2.5	10.4
Fecal Enterococci (CFU/100 mL)	1.50×10^6	6.34×10^5	7.06×10^5	5.11×10^5	NA	1.0	4.9	1.8
Cryptosporidium parvum (cyst/L)	148	240	215	304	NA	–	–	–
Giardia lamblia (cyst/L)	370	351	272	491	125–300	–	–	–
E. coli (CFU/100 mL)	1.04×10^6	3.12×10^6	2.56×10^6	1.52×10^6	NA	–	11.8	22.7
Extended Spectrum Beta-Lactamase (ESBL) (CFU/100 mL)	1.00×10^6	2.67×10^5	1.73×10^5	2.49×10^5	NA	–	50	1.5
Klebsiella pneumoniae Carbapenemase (KPC) (CFU/100 mL)	–	–	1.88×10^4	–	NA	–	–	–
Vancomycin resistant enterococci (VRE) (CFU/100 mL)	–	1.00×10^4	2.91×10^4	5.87×10^3	NA	–	–	157.6

[a] Canal serves community Group A.
[b] Canal serves community Group B.
[c] Well serves community Group C.

Mezquital Valley in the rainy season estimated two-week diarrheal prevalence of 29% and 23% among exposed and unexposed children, respectively. After using our conversion factor of 0.75, the prevalence this study would be expected to measure using one-week recall would be 22% and 17% among exposed and unexposed children (Table 7). The 2001 study conducted during the dry season estimated two-week prevalence of 19% and 14% among exposed and unexposed children, respectively. The converted prevalence estimates for this study had it used one-week recall are 14% and 11% among exposed and unexposed children.

4. Discussion

In our study, diarrheal prevalence over the prior week was estimated to be twice as high among children living in communities irrigating with untreated wastewater (10%) compared to unexposed communities (5%). After converting two-week diarrheal prevalence to one-week prevalence with our formula, we estimated one-week prevalence for exposed and unexposed in earlier studies as 22% and 17% in the rainy season and 14% and 11% in the dry season. These results suggest that overall diarrheal prevalence in the exposed communities decreased by about 50% since the seminal studies of Blumenthal and Cifuentes were conducted in the early 1990s (from approximately 22%–10%). However, at the same time, we found a considerably stronger effect of wastewater exposure on diarrheal rates (PR = 2.32) compared to the earlier studies (OR = 1.33). The prevalence ratio increase is likely even stronger than these numbers suggest considering that odds ratios tend to be overestimates of relative prevalence (Zocchetti et al., 1997). Thus, our study suggests that overall diarrheal disease burden has decreased in these communities over the past 25 years, but the relative influence of wastewater exposure on diarrhea has likely increased. One possible explanation is that other risk factors for diarrheal disease, such as poor hygiene or inadequate sanitation, might have decreased over this time, while the amount of wastewater exposure in these communities has not changed. This finding is supported by the level of contamination we found in wastewater that has remained high since earlier Mezquital studies.

The two-fold increase in prevalence of diarrheal disease associated with wastewater exposure that we found is consistent with estimates from other previous studies that compared diarrheal prevalence between wastewater-exposed and unexposed communities. The most comparable studies found diarrheal prevalence to be 2.0 to 2.7 times higher among exposed communities (Agunwamba, 2001; Ensink et al., 2006; Feenstra et al., 2000; Gumbo et al., 2010).

4.1. Exposure pathways

When we limited our analysis to households with at least one field worker, we found a somewhat weaker, non-statistically significant association between wastewater exposure and diarrheal disease. If direct engagement in wastewater irrigation were a strong risk factor for diarrhea in the Mezquital Valley, we would expect to find a stronger association in this group compared to the association found when using the entire population. In contrast, our results suggest that direct participation in agriculture is likely not the most important exposure pathway, though our study was not powered to observe a difference in this subsample. Although our finding may be due to small sample size, it is supported by previous

Table 7
Comparison of these study results to previous studies conducted in the Mezquital Valley.

Study	Season	Two-week prevalence as reported (exposed vs. unexposed)	One-week diarrheal prevalence (exposed vs. unexposed)	OR/RR (95% CI)[b]	Covariates included in adjusted model
Cifuentes, 1998	Rainy season, 1992	29% vs. 23%	22% vs. 17%[a]	1.33 (0.96, 2.18)	Boiling drinking water
Blumenthal et al., 2001	Dry season, 1991	19% vs. 14%	14% vs. 11%[a]	1.75 (1.10, 2.78)	Handwashing, roof material, number of bedrooms
This study	Rainy season, 2015	–	10% vs. 5%	2.31 (1.00, 5.31)	Education, average age of children under five, number of children under five in household
This study, limited to homes with field worker	Rainy season, 2015	–	10% vs. 5%	1.81 (0.75, 4.38)	Education, average age of children under five, number of children under five in household

[a] Two-week diarrheal prevalence from previous studies was multiplied by 0.75 to convert to estimated one-week prevalence.
[b] OR's are not affected by conversion to one-week prevalence, assuming rate of underreporting was not related to exposure.

findings that participation in wastewater irrigation was not a risk factor for disease in communities reusing wastewater (Ensink et al., 2005, 2006, Pham-Duc et al., 2011, 2013, 2014; Trang et al., 2007a). These results indicate that participation in wastewater irrigation may not be the most important route of exposure leading to more disease in these communities. Nonetheless, we did find that wastewater remains an increasingly important contributor to diarrheal disease in the overall community. Other significant exposure pathways that affect the general population in communities engaged in wastewater reuse might include community contamination and contact with exposed crops.

4.2. Stool and environmental samples

Microbial analyses of stool, water, and dust samples demonstrated contamination among both exposed and unexposed communities. Fecal contamination was found in well water and unexposed households, but samples from exposed communities showed higher overall contamination. We found similar levels of fecal contamination in wastewater samples as those in earlier Mezquital studies, and even slightly higher concentrations of *Giardia* cysts (272–491 cysts/liter) than those found previously (125–300 cysts/liter) (Cifuentes et al., 2000). Wastewater quality and contamination found in household water and dust further support that wastewater exposure might be influencing higher diarrheal prevalence in these communities.

4.3. Future studies and caveats to study design

A larger longitudinal study is needed to test the hypotheses generated from this study and comparisons made to past studies. In both a cross-sectional and longitudinal study, the comparability between communities exposed and not exposed to wastewater may be an issue if there are any unmeasured confounding variables. Although the communities in this study are similar on all measured variables, such as sociodemographic characteristics, those exposed to wastewater are also exposed to other environmental factors not included in this analysis that may affect their health. In particular, wastewater-exposed communities in this study have more industrial activity than control communities, including the presence of an industrial park and a large oil refinery near communities in Group A. Increased number of stool and household environmental samples would allow for pathogen-specific analysis. Temporal sampling of wastewater quality and incident diarrheal cases would allow for more sophisticated time series analysis. With regards to comparisons with earlier studies in the Valley, we chose a different set of control communities, limiting the direct comparability to previous results. Our comparison group consists of communities using well water for irrigation that are more socioeconomically similar to the exposed communities but located further away geographically compared to the rainwater irrigating communities included in the original studies.

5. Conclusions

Our study highlights that 10 years after the publication of the WHO guidelines for wastewater reuse and 25 years after the seminal studies by Blumenthal and Cifuentes that informed them, communities in the Mezquital Valley still face high health risks due to wastewater exposure. Our review of recent epidemiologic evidence shows that this phenomenon is seen worldwide. The upcoming opening of a treatment plant in this area provides the opportunity to directly measure the effects of treatment on water quality and health that will have relevance to other urban areas with minimal sewage treatment. The Sustainable Development Goals (SDGs) for 2030 are focused on not only ensuring wastes are safely kept from the populations generating the waste, but also are sufficiently treated so that downstream communities are also protected. The Mexico City-Mezquital Valley system will be an important site to monitor over the coming years as an example of how these goals can be pursued.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2017.06.058.

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