Seasonal and gender impacts on fecal exposure trends in an urban slum

Min-Li Chua[a], Md. Nazmul Ahsan [b], Akira Sakai[c], Shigeo Fujii IWA[a], Shotaro Goto[a], Michiya Kodera[a] and Hidenori Harada IWA [d],*

[a] Graduate School of Global Environmental Studies, Kyoto University, Yoshida-honmachi, Sakyo-ku, Kyoto 606-8501, Japan
[b] Life Science School, Khulna University, Gallamari, Khulna 9208, Bangladesh
[c] University of Marketing and Distribution Sciences, 3-1 Gakuen-Nishimachi, Nishi-ku, Kobe 651-2188, Japan
[d] Graduate School of Asian and African Area Studies, Kyoto University, 46 Yoshida-shimoadachicho, Sakyo-ku, Kyoto 606-8501, Japan
*Corresponding author. E-mail: harada.hidenori.8v@kyoto-u.ac.jp

MNA, 0000-0003-0959-3247; HH, 0000-0002-7685-7751
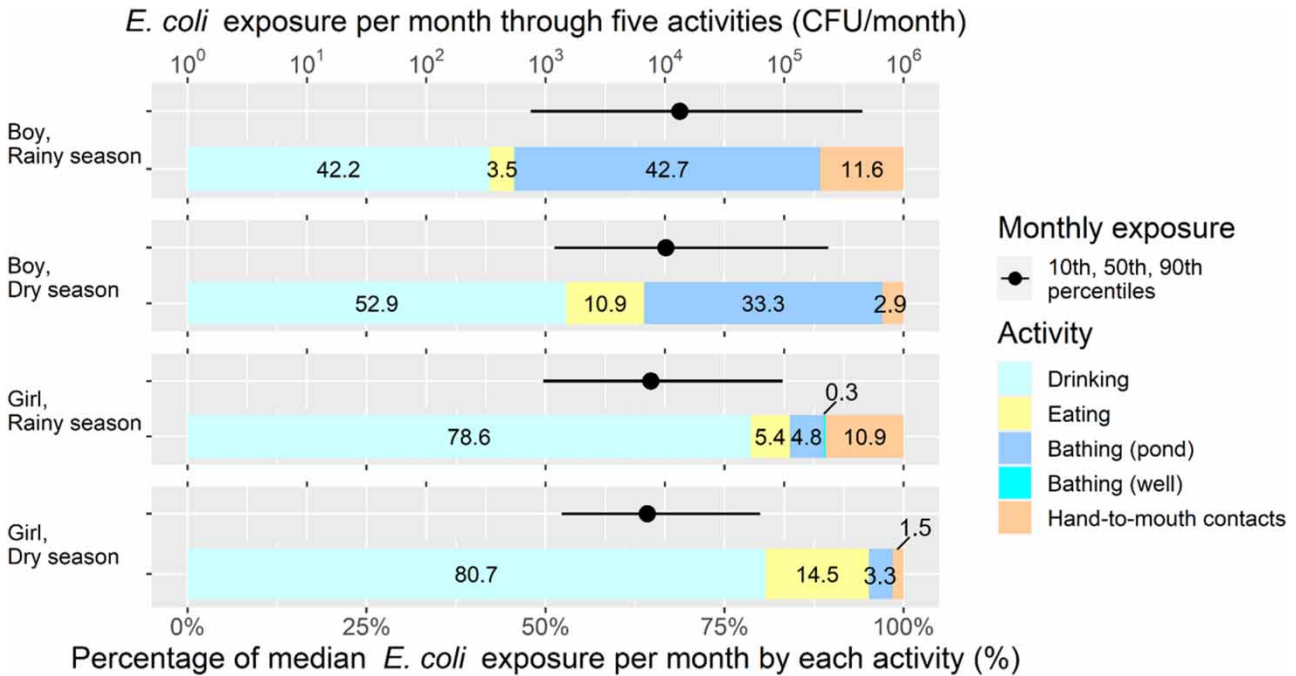
ABSTRACT

Seasonal and gender impacts have not been well considered in fecal exposure assessment, especially in low- and middle-income countries. This study examined the seasonal and gender impacts on fecal exposure trends in children through daily living activities in an urban slum in Bangladesh. We determined *Escherichia coli* concentrations in seven types of environmental samples ($n = 232$) and the activity data of children via diary recording, questionnaires, and interview surveys. Daily and monthly exposures were stochastically estimated for drinking, eating, pond bathing, well bathing, and hand-to-mouth contact. Of the five pathways, pond bathing and drinking contributed a large part of the daily and monthly exposure. Significant seasonal differences were observed in daily exposures for bathing, which were higher in the rainy season (2.59×10^2 CFU/day for boys and 6.19×10^{-1} CFU/day for girls) than in the dry season (1.69×10^2; 4.30×10^{-2}), because of longer pond bathing time and more contaminated bathing water in the rainy season. In contrast, eating had significantly higher exposure in the dry season (3.71×10; 3.22×10) than the rainy season (1.50×10; 1.24×10) due to the higher dish contamination. Significantly higher daily exposure was observed in the bathing for boys than girls, as boys spent longer time for bathing at a heavily contaminated pond.

Key words: children, environmental media, *Escherichia coli*, fecal exposure assessment, quantitative microbial risk assessment (QMRA)

HIGHLIGHTS

- Seasonal and gender impacts on fecal exposure were studied in a slum in Bangladesh.
- *Escherichia coli* levels of seven types of samples and activity data of children were collected.
- Fecal exposure of children through five pathways was modeled by seasons and gender.
- Drinking predominantly contributed to the exposure of girls regardless of seasons.
- Boys' preference to pond bathing caused the largest exposure in the rainy season.

GRAPHICAL ABSTRACT

E. coli exposure per month through five activities (CFU/month)

Percentage of median *E. coli* exposure per month by each activity (%)

INTRODUCTION

Despite progressive efforts to improve global health, diarrheal diseases remain a critical problem, especially among children in lower middle-income settings, mainly because of unsafe water, sanitation, and hygiene (WASH) (Troeger *et al.* 2018; Prüss-Ustün *et al.* 2019). Fecal contamination from WASH-poor settings is associated with an increase in the risk of diarrhea in children (Pickering *et al.* 2018; Qadi *et al.* 2019; Goddard *et al.* 2020). It is thus essential to evaluate fecal exposure of children in a living environment to identify effective methods for reducing diarrheal risks.

Quantitative microbial risk assessment (QMRA) is a framework that has been used to assess public health risks and suggest suitable interventions (WHO 2016); fecal exposure analysis is a part of QMRA that quantifies human exposure to fecal matter in a specific context. Along with high-income countries, fecal exposure analysis and analytic tools, including the Rapid Participatory Sanitation System Risk Assessment (RPSSRA) (Campos *et al.* 2015; Nayebare *et al.* 2020) and SaniPath (Wang *et al.* 2018; Amin *et al.* 2019; Raj *et al.* 2020), have also been utilized in low- and middle-income contexts. These studies have mainly described the cross-sectional exposure of a representative population group at a certain time and did not consider variability in time or in the population of the target community.

However, previous studies on fecal contamination, behavior, and diarrhea frequency have shown that seasonal and gender factors may produce variations in exposure trends. For example, in a review of developing country studies reporting both urban and rural findings, 15 of 26 studies found that drinking water sources were significantly more contaminated in the rainy season than in the dry season (Kostyla *et al.* 2015). Furthermore, in the rural United States, boys had more frequent environmental contact by hand than girls; however, girls had longer durations of environmental contact than boys (Beamer *et al.* 2008). These studies imply the potential impacts of seasonality and gender on exposure analysis. Although proper consideration of seasonality and gender might improve fecal exposure assessment, limited knowledge is available regarding the seasonal and gender impacts on fecal exposure trends, especially in slum communities including the most vulnerable populations.

Bangladesh has one of the most severe child mortalities and has a long history of overpopulation and inadequate WASH. Bangladesh has a subtropical monsoon climate and is reported to have distinct seasonal differences in environmental conditions (Khatun *et al.* 2016; Chowdhury *et al.* 2018; Mohsenipour *et al.* 2020) and human behavior (Rahman *et al.* 2018; Haque *et al.* 2020). Bangladesh also has social differences between boys and girls due to their gender-specific family roles

and religious practices (Ahmed & Sen 2018; Ferdous & Mallick 2019). The present study thus aimed to identify the potential impacts of seasons and gender on the exposure trends of children via daily living activities in slum settings. To achieve this goal, the following were conducted in an urban slum in Bangladesh: (1) evaluation of environmental contamination data regarding exposure media and behavioral data for various pathways with seasonal and gender-based contexts; (2) stochastic modeling of fecal exposure considering the seasonal and gender differences; (3) examination of seasonal and gender impacts on the exposure trends; and (4) sensitivity assessment of each input variable involved in the modeling.

METHODS

Study area

This study was carried out in a slum community, known as Camp 1, in Khulna City, Bangladesh. Khulna is the third largest city in the country, with an area of 50.6 km^2 and a population of 660,000 (Bangladesh Bureau of Statistics 2014). Camp 1 had a population of approximately 2,500 settled in 350 households (JADE 2016). There were 72 tubewells (12 for drinking and 60 for non-drinking purposes) and 146 latrines (36 community latrines and 110 private latrines); however, the facilities were poorly managed under excessive local demand (Harada *et al.* 2018).

Four meteorological seasons have been reported in Bangladesh: hot-humid pre-monsoon (March–May), humid-rainy monsoon (June–September), hot-dry post-monsoon (October–November), and cool-dry winter (December–February) (Khatun *et al.* 2016). For seasonal comparison in this study, the dry (or non-rainy) season was defined as the post-monsoon and winter months, whereas the rainy season was defined as the pre-monsoon and monsoon months.

Fecal contamination data collection

Samples were collected from households who consented to participate in this study during November–December in 2013 and 2015 for the dry season, and during September–October in 2013 and 2015 for the rainy season. The collected samples were as follows: deep tubewell water at point-of-use (POU) ($n = 36$ for dry season; $n = 42$ for rainy season) in kitchens, shallow tubewell water at the source (24; 13) where the residents usually used it for bathing, pond water at the source (20; 14) from two nearby ponds, outdoor soil (20; 15) at locations where children were found playing on the ground, inner surfaces of dishes and cups (14; 10) that were washed, dried, and kept in kitchens, cooked food (0; 14), and the hands of children. According to the Joint Monitoring Programme (JMP)'s classifications of WASH facilities (WHO & UNICEF 2018), the deep and shallow tubewells were categorized as improved water sources with aprons to divert spilled water away from the well, closed tube ends, and hand pumps. The two ponds were used for bathing, swimming, and washing. Public latrines were found connected directly to the ponds and livestock animals were moving freely beside the ponds with observable fecal matter scattered on the ground. Cooked food was sampled only during September–October 2015 with no contamination observed; it was assumed that there were no seasonal differences in food practices, and thus no food contamination was assumed for both the dry and rainy seasons. Hand samples were collected from the left and right hands separately from children aged 6–11 years. This age group was selected because at this age, children begin to actively engage in vigorous activities in daily life (US EPA 2005). The hand samples were categorized into washed and non-washed conditions, with and without seasonal variation, respectively. Washed hands ($n = 10$ boys and 11 girls, right hand only) represented the hands that were washed when requested by the researcher to behave as if they were preparing to take a meal in their regular daily life. The children had their meals using their right hands. They washed their hands using shallow tubewell water with or without soap, depending on their daily practice; out of 21 washed hand samples, 10 were washed with soap by six boys and four girls. For non-washed hands ($n = 28$ boys and 28 girls in the dry season, 30 boys and 32 girls in the rainy season; left and right hands), children were requested to not wash their hands before sampling.

Water and food samples were collected in sterilized sampling bags, and surface samples were collected using swab test kits (Pro-media ST25 PBS, ELMEX, Japan). All samples were transported on ice to the laboratory within 4 h. The *Escherichia coli* test was then carried out through membrane filtration (0.45 μm pore size; EMD Millipore Microfil V Filtration Device, Fisher Scientific, USA) and agar cultivation (XM-G agar, Nissui, Japan) with incubation at 37 °C for 22 h. The *E. coli* concentration of each collected sample was obtained by counting blue colonies from the *E. coli* cultures.

Activity data collection

Activity data were collected from households who consented to participate in this study. First, general activity trends were obtained via diary recording of children's activities by requesting six adult family members of individual households ($n = 3$

boys and 3 girls; aged 6–11) to determine the types of activities, duration of activities, types of objects contacted, and the frequency of hand-to-mouth contacts observed with each activity in a day. Second, five major types of activities were selected based on the data obtained above: drinking, eating, well bathing, pond bathing, and hand-to-mouth contact during times excluding the former four activities and sleeping. Third, further detailed activity data were collected using two surveys. One of the surveys was a questionnaire for mothers ($n = 30$), as they were responsible for domestic duties: (1) daily frequency and amount of water usage in drinking and (2) daily frequency and amount of eating. According to the on-site observations, pond and well bathing activities by girls resembled a showering behavior using a water bucket, that is, by pouring water on themselves, whereas pond bathing activities by boys resembled swimming behavior. Thus, the second survey was an interview survey for boys and girls separately, regarding their bathing activities ($n = 10$ boys and 10 girls aged 6–11), including the water source, daily frequency, and duration of pond and well bathing, in both the dry and rainy seasons. Seasonal variation was excluded from the activity data of drinking, eating, and hand-to-mouth contact, as the respondents did not recognize any seasonal differences. Gender variation was excluded from the activity data of drinking and eating, as mothers did not recognize any gender-related differences. The details regarding the methodology of activity data collection are listed in Supplementary Material, Table S1.

Data fitting into probability density functions

E. coli concentration data and activity data were fitted into a probability density function as summarized in Supplementary Material, Tables S2 and S3, respectively. Briefly, *E. coli* concentration data were all fitted to log-normal distributions, as described previously (Wymer & Wade 2007; Pleil *et al.* 2014). As no *E. coli* was detected in cooked food samples in this study ($n = 14$), cooked food was assumed to be uncontaminated and was therefore excluded from the subsequent assessment. Water consumption per capita per day was fitted to a log-normal distribution, as recommended by previous studies (Roseberry & Burmaster 1992; US EPA 2011). Around 10 positive samples are necessary to precisely estimate distribution parameters (Kato *et al.* 2013). For data with *n* equal and less than 10, resampling was performed with replacement, as recommended by a past study (Julian *et al.* 2018). For activity data with more than 60% repeated observed values, resampling was performed with replacement, whereas data with equal and less than 60% repeated observed values were fitted into a normal distribution as all of them showed $p > 0.05$ by the Shapiro–Wilk normality test. All data fitting was based on the maximum-likelihood method using the R package, 'fitdistrplus' (R Core Team 2016).

EXPOSURE MODEL

Exposure pathways and their associated media

This study regarded each activity as a single pathway. Of the five pathways in total, two were dietary pathways, including drinking (media involved: drinking water and drinking cup) and eating (hand and dish), and three were non-dietary pathways, including pond bathing (pond water), well bathing (well water), and hand-to-mouth activity during the times excluding the former four activities and sleeping (hand surface). Of the three non-dietary pathways, exposure to the former two pathways occurred through accidental ingestion of water, and that to the latter pathways occurred through hand-to-mouth contact. Each calculation is explained as follows.

Daily and monthly exposure calculation

Daily and monthly fecal exposures through each potential exposure pathway by genders (boys or girls) and seasons (dry or rainy season) were calculated stochastically. Exposure factors, or unit intake amounts of exposure media (Supplementary Material, Table S4), and other parameters were obtained from previous studies (Supplementary Material, Table S5). The number of days when each activity was performed in a month is summarized in Supplementary Material, Table S6. Of the five pathways, exposure through drinking was calculated using Equation (1), by assuming that all *E. coli* present on the inner cup surface are ingested by the child, as follows:

$$E_{day,i,g,s} = [(C_{stored,s} \cdot A_{drink}) + (C_{cup,s} \cdot c_{cup} \cdot F_{drink})] \tag{1}$$

where $E_{day,i,g,s}$ is the daily exposure amount through activity/by gender g and season s (CFU/day), $C_{stored,s}$ is the *E. coli* concentration of stored drinking water by season s obtained from a probabilistic density function (PDF) defined (CFU/mL), A_{drink} is the amount of drinking water intake (mL/capita/day), $C_{cup,s}$ is the *E. coli* concentration of inner cup surface by season s

obtained from a PDF defined (CFU/cup), c_{cup} is the unit conversion factor in terms of cup surface (1 cup/event), and F_{drink} is the frequency of drinking events per day (events/day).

For the eating pathway, *E. coli* was not detected in any of the food samples in this study ($n = 14$). Exposure through this pathway was calculated using Equation (2) with the following assumptions: (1) no *E. coli* is present in food, (2) all *E. coli* on the plate and on the washed hand are ingested through eating, and (3) only one plate is used for each eating event:

$$E_{day,i,g,s} = [(C_{washed-hand,g} \cdot c_{eat,washed-hand}) + (C_{dish,s} \cdot c_{eat,dish})] \cdot F_{eat} \tag{2}$$

where $C_{washed-hand,g}$ is the *E. coli* concentration of washed hand surface by gender g obtained from a PDF defined (CFU/hand), $c_{eat,washed-hand}$ is the unit conversion factor in terms of washed hand surface (1 hand/event), $C_{dish,s}$ is the *E. coli* concentration of dish surface by season s obtained from a PDF defined (CFU/dish), $c_{eat,dish}$ is the unit conversion factor in terms of dish surface (1 dish/event) and F_{eat} is the frequency of eating events per day (events/day).

Exposure through the pathways of well bathing and pond bathing was calculated using Equation (3), by assuming that (1) pond bathing by boys follows the exposure factor of swimming and (2) pond bathing by girls and well bathing by boys and girls follow the exposure factor of showering:

$$E_{day,i,g,s} = C_{j,s} \cdot c_{bath,j} \cdot U_{bath,j} \cdot t_{bath,g,s} \cdot F_{bath,g,s} \tag{3}$$

where $C_{j,s}$ is the *E. coli* concentration of bathing medium j by season s obtained from a PDF defined (CFU/amount of medium j), $U_{bath,j}$ is the exposure factor, that is, unit intake of medium j for bathing activity per hour (amount of medium j/h), $t_{bath,g,s}$ is the time duration of bathing activity by gender g and season s per event (h/event), and $F_{bath,g,s}$ is the frequency of bathing activity by gender g and season s per day (events/day).

Exposure through hand-to-mouth contact during the times excluding the former four activities and sleeping was calculated using Equation (4) by assuming that only one hand is used for hand-to-mouth contact:

$$E_{day,i,g,s} = C_{hand,g,s} \cdot c_{htm,hand} \cdot F_{htm,g} \cdot S_{htm} \cdot T_{htm} \cdot t_{htm} \tag{4}$$

where $C_{hand,g,s}$ is the *E. coli* concentration of non-washed hand surface by gender g and season s obtained from a PDF defined (CFU/hand), $c_{htm,hand}$ is the unit conversion factor in terms of the non-washed hand surface (1 hand/contact), $F_{htm,g}$ is the frequency of hand-to-mouth contact by gender g per non-drinking, non-eating, non-bathing, and non-sleeping hours (contact/h), S_{htm} is the percentage of mouth area contacted by hand per area of the hand surface (dimensionless), T_{htm} is the transfer efficiency of *E. coli* from the hand to mouth (dimensionless), and t_{htm} is the time duration of non-drinking, non-eating, non-bathing, and non-sleeping hours per day (h/day).

The total monthly exposure amount for a child was calculated using Equation (5) with the following assumptions: (1) he or she only performs either pond bathing or well bathing in a day; (2) drinking, eating, and hand-to-mouth contacts are done every day; and (3) there are 30 days in a month:

$$E_{T,g,s} = \sum_{i,g,s} E_{day,i,g,s} \cdot d_{i,g,s} \tag{5}$$

where $E_{T,g,s}$ is the total exposure amount of all activities by gender g and season s over a month (CFU/month), and $d_{i,g,s}$ is the number of days conducting activity i by gender g and season s over a month (day/month).

Thus, using Equations (1)–(5), fecal exposure was assessed stochastically using Monte Carlo simulations (100,000 iterations).

Sensitivity analysis

Sensitivity analysis was performed for each parameter involved in the exposure assessment. The approach of this analysis was modified based on previous studies (Xue *et al.* 2006; Julian *et al.* 2018) by comparing the output exposure amount when a particular parameter was set at either the 10th (p10), 50th (p50), or 90th (p90) percentile values, whereas the others were set at p50 as the baseline. For instance, an output value of the total exposure amount was produced when one selected parameter was set at its p10 and the rest were at p50; this was then repeated for the p50 and p90 scenarios. The ratios of output

values for the p10:p50, p50:p90, and p10:p90 scenarios were obtained and compared to identify the top three most sensitive parameters in the assessment.

Statistical tests

The Mann–Whitney test was used to determine significant differences ($p < 0.05$) between two groups. For the Monte Carlo simulation results, significant differences ($p < 0.05$) between two groups were identified using the Monte Carlo permutation test (Šmilauer & Lepš 2014). Briefly, the null hypothesis states that the response data are dependent on the explanatory variables; the smaller the expected value, the stronger is the evidence against the null hypothesis. Thus, the p-value was calculated using Equation (6), as follows:

$$p = \frac{n_x + 1}{N + 1} \tag{6}$$

where n_x is the number of iterations when the expected value was lower than the observed value. N is the total number of iterations in generating the expected values.

RESULTS AND DISCUSSION

E. coli contamination levels in environmental samples

E. coli was detected in 174 of 232 environmental samples from the six sample types (Table 1). Notably, deep tubewell water (POU) stored in households was significantly more contaminated than shallow tubewell water at the wells (point-of-use, POC as well as POU) ($p = 0.02$), even though the former was used for drinking and the latter was used for non-drinking purposes. Previous studies have suggested that contamination of drinking water occurs between the POC and the POU (Rufener et al. 2010; Harada et al. 2018). Deep tubewell water stored in households (POU) is potentially contaminated between the POC and the POU, resulting in higher contamination than the shallow tubewell water in the wells.

At the site, 70.8% of cups and 54.2% of dishes in the household kitchens were E. coli positive; 54.1% of shallow tubewell water, which was used for washing the cups and dishes, was contaminated (Table 1). It is possible that the E. coli contamination from the cups and dishes was transferred from the contaminated well water, as indicated by previous studies in urban India (Bhaskar et al. 2004) and urban Burkina Faso (Barro et al. 2006).

The median E. coli concentrations of soil samples, and cup and dish surfaces were 3.29 $\log_{10}$ CFU/g, 0.02 and 0.30 $\log_{10}$ CFU/media, respectively. The latrines at the study site had direct discharge to open drains that were clogged with dirty water and solid waste. Livestock animals moved freely, and their feces were scattered on the ground. These poor drainage and animal waste management conditions may be associated with soil contamination.

Seasonal and gender differences in E. coli concentration

The E. coli concentrations of six types of samples, shown in Table 1, were further analyzed for seasonal trends, as shown in Figure 1. The E. coli concentrations of water samples in the dry and rainy seasons were, respectively, 0.08 and 0.53 $\log_{10}$ CFU/100 mL (median) for deep tubewell water stored in households, ND (not detected) per 100 mL and 0.56 $\log_{10}$ CFU/ 100 mL for shallow tubewell water at the wells, and 3.62 and 3.74 $\log_{10}$ CFU/100 mL for pond water. Among the three water types, shallow tubewell water at the wells was significantly more contaminated in the rainy season ($p < 0.001$). E. coli

Table 1 | Summary of E. coli concentration in environmental samples

Sample type	Unit	n (+)	Median	p10–p90
Deep tubewell water stored in households (POU; drinking)	$\log_{10}$ CFU/100 mL	78 (55)	0.39	ND–1.58
Shallow tubewell water at wells (POU; bathing and cleaning)	$\log_{10}$ CFU/100 mL	37 (20)	−0.22	ND–0.90
Pond water at a pond (POU; bathing)	$\log_{10}$ CFU/100 mL	34 (34)	3.64	3.26–4.88
Outdoor soil	$\log_{10}$ CFU/g	35 (35)	3.29	2.51–4.52
Dish	$\log_{10}$ CFU/medium	24 (17)	0.30	ND–1.84
Cup	$\log_{10}$ CFU/medium	24 (13)	0.02	ND–1.50

POU, point of use; n (+), sample size (positive samples); p10–p90, range of 10th–90th percentiles, ND, not detected.
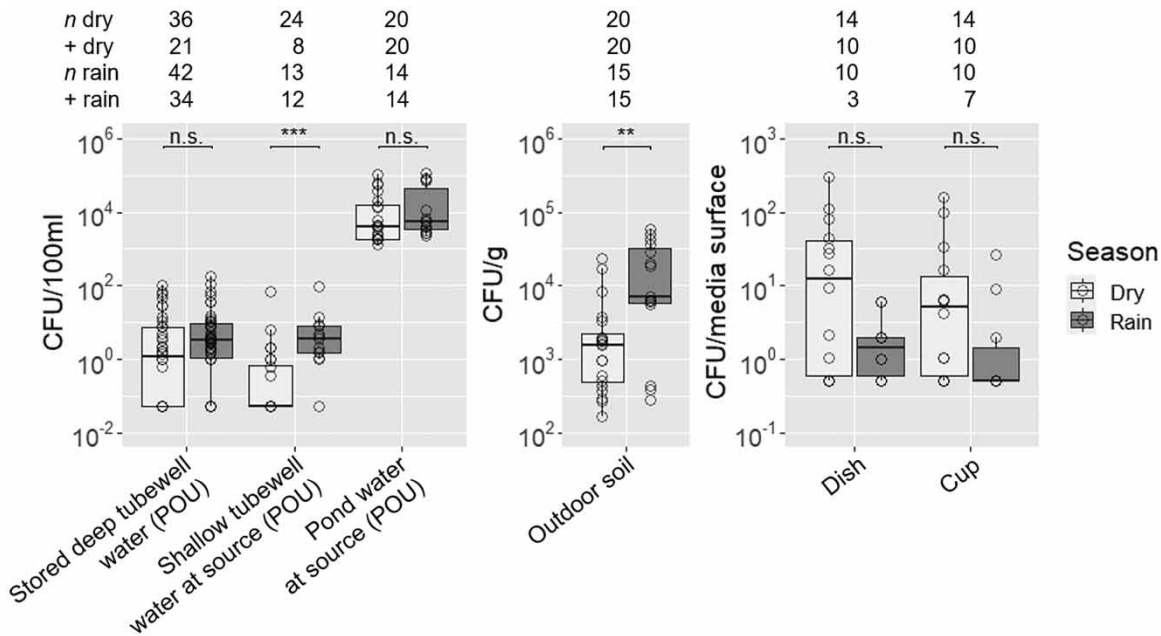
Figure 1 | *E. coli* concentrations of six environmental media in both the dry and rainy seasons. The box plots show the median and inter-quartile ranges. Point plots show the *E. coli* concentrations. The numbers at the top of the figure show the total sample number (*n*) and the *E. coli*-positive sample number (+) in the dry and rainy seasons. The Mann–Whitney test was performed to compare two groups. ND data were set to half of their detection limits in this figure. POU, point of use; n.s. represents no significant difference. **$p < 0.01$, ***$p < 0.001$.

concentrations of soil and surface samples in the dry and rainy seasons were, respectively, 3.19 and 3.85 $\log_{10}$ CFU/g for outdoor soil, 1.11 and 0.18 $\log_{10}$ CFU/medium for dishes, and 0.72 $\log_{10}$ CFU/media and not detected (ND) per medium for cups. Shallow tubewell water ($p < 0.001$) and outdoor soil ($p < 0.01$) were significantly more contaminated during the rainy season.

Similar to the shallow tubewell water and outdoor soil in the present study, higher *E. coli* concentrations in the rainy season were also observed in previous studies from the rural and urban areas of developing countries (e.g., van Geen *et al.* 2011; Bauza *et al.* 2017; Osiemo *et al.* 2019). As the potential reasons for this seasonal trend, Kwiringira *et al.* (2016) and Osiemo *et al.* (2019) suggested that surface runoff during the rainy season spread *E. coli* from clogged drains to wider environments. The poor drainage observed in the present study may have resulted in higher levels of fecal contamination in the rainy season than in the dry season.

For hand samples, the median *E. coli* concentration was 0.68 $\log_{10}$ CFU/hand on non-washed hands, 0.48 on hands washed without soap, and 0.48 on hands washed with soap. The *E. coli* concentration on the left and right hands was, respectively, 0.50 and 0.54 $\log_{10}$ CFU/hand. There were no significant differences in the washing conditions and in the left and right hands (Supplementary Material, Figures S1 and S2). After washing, the hands were still contaminated with *E. coli*. The contaminated shallow tubewell water used for handwashing (Figure 1) implies cross-contamination, as discussed by Taulo *et al.* (2009) and Aihara (2014).

For seasonal and gender trends on non-washed hands (Figure 2), the median *E. coli* concentrations were 0.20 and 1.03 $\log_{10}$ CFU/hand in the dry and rainy seasons, and 0.90 and 0.42 $\log_{10}$ CFU/hand for boys and girls, respectively. The concentrations were significantly different by seasons ($p < 0.05$), but not by genders (Supplementary Material, Figure S3). Seasonal differences in soil contamination may have affected the hand contamination of children, as previous studies discussed the association between hand-media contact and contamination of the surrounding environment (Pickering *et al.* 2011; Julian *et al.* 2018). Increased hand contamination may be caused by the more contaminated environment in the rainy season. Higher hand contamination in the rainy season could potentially cause higher contamination of stored tubewell water in the rainy season when handling the storage and collection of water.

Seasonal and gender differences in activity

The time duration of bathing activities for boys and girls was, respectively, 0.33 and 0.08 (median) hour/day in pond bathing, and 0.07 and 0.33 hours/day in well bathing (Figure 3). The duration of bathing between boys and girls was significantly different in pond bathing ($p < 0.001$) and well bathing ($p < 0.001$).
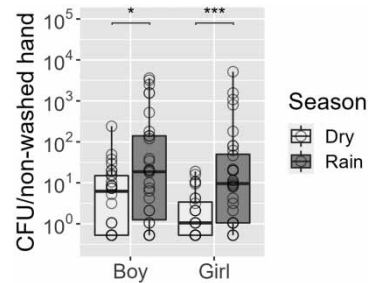
Figure 2 | *E. coli* concentrations of non-washed hands for boys and girls in the dry and rainy seasons. Boxplots show the median and interquartile ranges. Point plots show the *E. coli* concentrations. ND data were set to half of their detection limit in this figure. The Mann–Whitney test was used to compare two groups. $*p < 0.05$, $***p < 0.001$.

The boys mainly performed pond bathing. The boys' preference for pond bathing may be associated with avoiding the waiting time for well water collection. Girls mainly performed well bathing, possibly because of the social aspects restricting female Muslims from going to public areas (Balk 1997); thus, they preferred to bathe in a closed environment surrounded by walls near the well. Through bathing activities, boys could ingest more *E. coli* due to heavy contamination of pond water compared to girls who bathed with less contaminated water from a shallow tubewell (Figure 1). There were no significant differences in the seasonal trends of the main bathing activities of each gender (Supplementary Material, Figure S4).

The medians of hand-to-mouth contact frequency were 5.09 contacts/hour for boys and 3.0 contacts/hour for girls. There were no significant differences in genders for both hand-to-mouth contact frequencies (Supplementary Material, Figure S5). These frequencies were of the same magnitude as those for children in a previous study in the United States, 4.8 (AM) contact/hour (Wilson *et al.* 2020). The higher frequency of hand-to-mouth contact in the present study may result in greater ingestion of *E. coli* from hands that were potentially contaminated by the living environment.

Exposure trends

Daily *E. coli* exposure was estimated for boys and girls in the dry and rainy seasons based on a typical daily scenario (Figure 4). A notable gender difference was observed with daily exposure through bathing (Figure 4). Boys in both dry and rainy seasons had more daily exposure through bathing than girls (dry season: 1.69×10^2 CFU/day for boys and 4.30×10^{-2} CFU/day for girls, $p < 0.05$; rainy season: 2.59×10^2 and 6.19×10^{-1} CFU/day, $p < 0.05$). This gender difference in bathing can be explained by the difference in the bathing location by genders in the typical daily scenario: pond bathing for boys and well bathing for girls. Pond water was significantly more contaminated than shallow tubewell water in the dry and rainy seasons, respectively (Figure 1). This indicated that boys' preference for pond water resulted in greater exposure through bathing compared to girls. In other words, girls had significantly mitigated exposure through bathing by choosing a safer water source, i.e., well water, for bathing.

Significant seasonal differences were also observed with daily exposure through bathing and eating (Figure 4). Daily exposures through bathing in the rainy season for both boys and girls, as mentioned above, were higher compared to
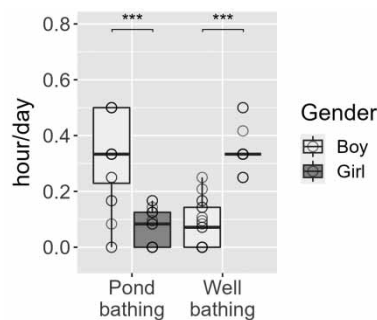

Figure 3 | Daily duration of pond bathing and well bathing for boys and girls. Boxplots show the median and interquartile ranges. Point plots show the daily durations. The Mann–Whitney test was used to compare two groups. $***p < 0.001$.
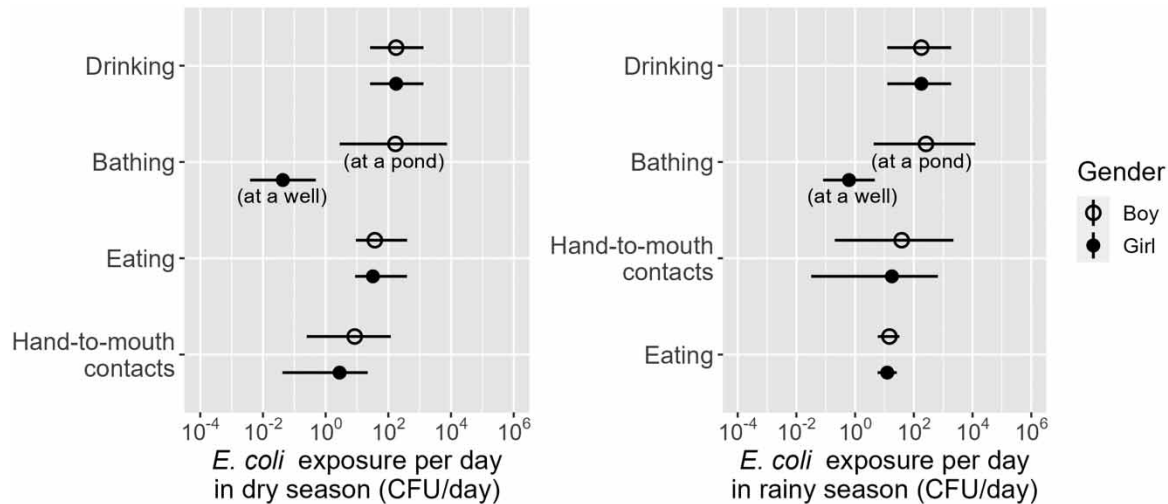
Figure 4 | Daily *E. coli* exposure for boys and girls in a typical scenario for each season-based transmission pathway with plots representing the 10th, 50th, and 90th percentiles of each activity. The pathways represent all potential exposure pathways for boys and girls. In this scenario, the bathing activity for boys and girls was set as pond bathing and well bathing, respectively.

those in the dry season (boys: $p < 0.05$; girls: $p < 0.001$). This was mainly caused by seasonal differences in the contamination of bathing media. *E. coli* concentrations of shallow tubewell water and pond water in the rainy season were higher than those in the dry season (shallow tubewell water: $p < 0.001$; pond water: not significant) (Figure 1). In contrast to the seasonal trends of daily exposure through bathing, daily exposures through eating in the dry season for both boys and girls were higher compared to that in the rainy season (boys: 3.71×10 CFU/day in the dry season and 1.50×10 CFU/day in the rainy season, $p < 0.001$; girls: 3.22×10 and 1.24×10 CFU/day in the dry and rainy season, respectively, $p < 0.001$). This may be associated with more dish contamination in the dry season than in the rainy season, although not significant (Figure 1). As we did not collect washed hand samples in the two seasons separately, we could not discuss the impact of seasonal differences caused by hand contamination.

The total monthly *E. coli* exposure was estimated for boys and girls in the dry and rainy seasons based on the number of days with each activity performed in a month (Figure 5). The total monthly exposures were, in a decreasing order, 1.33×10^4 CFU/month (median) for boys in the rainy season, 1.02×10^4 CFU/month for boys in the dry season, 7.62×10^3 CFU/month for girls in the rainy season, and 7.13×10^3 CFU/month for girls in the dry season. The major pathways of monthly exposure differed by genders. For example, in the rainy season, the exposure pathways for boys were bathing (42.7% of total monthly exposure by median), drinking (42.2%), and hand-to-mouth contact (11.6%), whereas those for girls were drinking (78.6%), hand-to-mouth contact (10.9%), and eating (5.4%).

In the monthly exposure trends, pond bathing and drinking contributed a large proportion of exposure in boys (42.7 and 42.2% in the rainy season; 33.3 and 52.9% in the dry season, respectively), whereas drinking predominantly contributed to the monthly exposure in girls (78.6 and 80.7% in the rainy and dry seasons, respectively). The less contribution by drinking in boys was caused by the larger contribution by bathing, especially in the rainy season. This was caused by boys' preference for bathing at pond (Figure 3), of which water was heavily contaminated (Figure 1). Thus, major pathways in monthly exposure changed due to the activity preference difference in genders as explained in the daily exposure analysis (Figure 4).

In the rainy season, fecal contamination in environmental media, especially in shallow tubewell water, was higher than that in the dry season. This seasonal difference in the contamination of environmental media resulted in higher exposure of a child during the rainy season, particularly through bathing activities. These bathing activities also resulted in gender differences in the exposures due to differences in bathing preferences and duration of activities. In short, the preference and intensity of bathing activities for boys and girls caused gender differences, whereas the characteristics of fecal contamination of environmental media caused seasonal differences in exposure.

The impacts of seasonal and gender differences in exposure suggest approaches to reduce fecal exposure. Drinking contributed the most in exposures of boys in the dry season and girls regardless of the season. Thus, it is recommended to keep
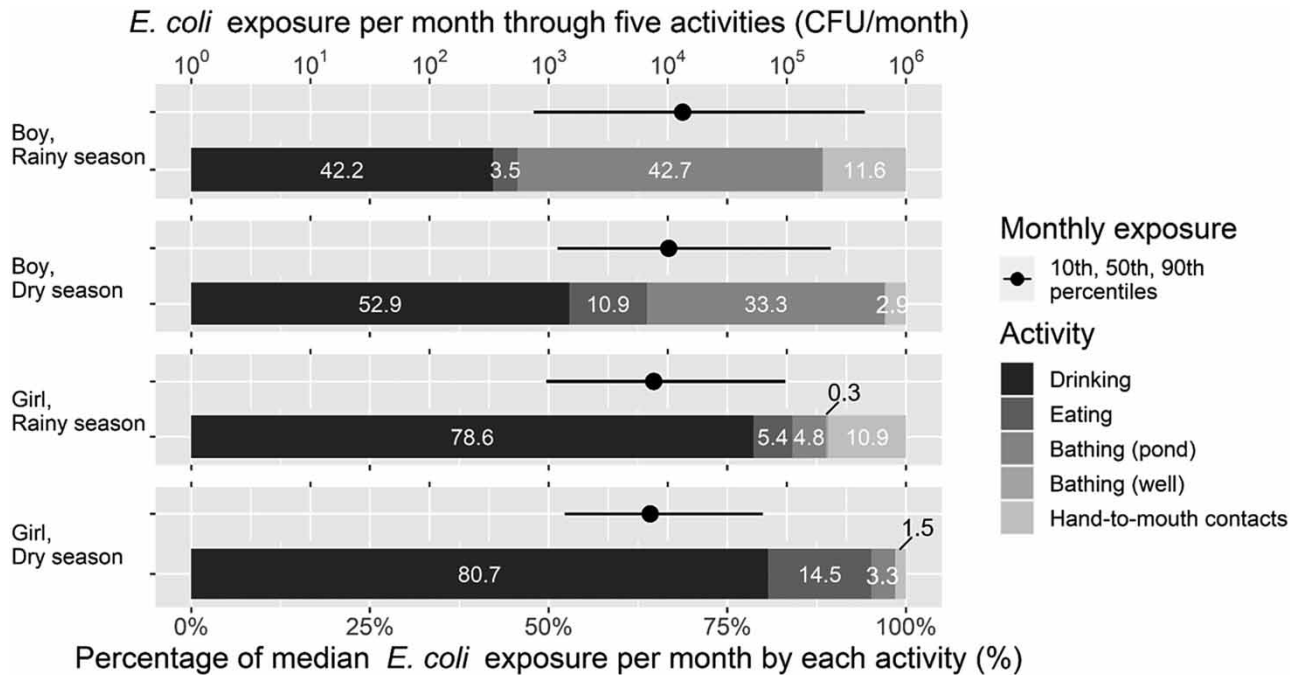
Figure 5 | Monthly *E. coli* exposure and percentage of median monthly *E. coli* exposure by each activity for boys and girls in the dry and rainy seasons.

drinking cups clean before use. Furthermore, handwashing and good storage practices of water should be considered to prevent potential contamination between the POC and the POU. Pond bathing was another major pathway for boys. As boys preferred bathing at a pond, protection of pond water or an alternative cleaner pond for bathing could be suggested.

Sensitivity analysis of monthly exposure

Sensitivity analysis was conducted on each parameter included in the monthly exposure analysis for boys in the rainy season, which was the highest exposure scenario (Table 2). Based on both p50:p10 and p90:p50 categories, the three most sensitive parameters for p50:p10 were the exposure factor of pond bathing, fecal contamination of pond water, and the daily duration of pond bathing, and those for p90:p50 were the exposure factor of pond bathing, fecal contamination of pond water, and fecal contamination of the hand surface.

Table 2 | Sensitivity analysis for exposure assessment of boys in the rainy season

| | Input value (percentile) | | | Output ratio of the total monthly *E. coli* exposure at two percentiles (ratio) | | | |
| | | | | (p50:p10) | | (p90:p50) | |
Parameter	(p10)	(p50)	(p90)	Rank	Value	Rank	Value
Exposure factor of pond bathing	0.4	9.9	262	1	2.53	1	17.12
Fecal contamination of pond water	1,618	9,931	59,568	2	2.12	3	4.15
Daily duration of pond bathing for boys	0.2	0.3	0.5	3	1.65	5	1.40
Daily frequency of pond bathing for boys	0.5	0.9	1.3	4	1.40		
Fecal contamination of hand surface for boys	0.5	19.7	805	5	1.18	2	7.34
Fecal contamination of stored deep tubewell water	0.1	2.5	43.4			4	3.52

Input variables were selected as either the 10th percentile (p10), 50th percentile (p50), or 90th percentile (p90) values individually to produce the output of total daily *E. coli* exposure. The ratios of the output values were then ranked by the top five highest ratio values, representing the five most sensitive parameters in the assessment. Sensitivity analysis for exposure assessment of boys in the dry season, and girls in the dry and rainy seasons is shown in Supplementary Material, Tables S8, S9, and S10, respectively.

Input variables related to pond bathing were the most sensitive in the exposure analysis for boys in the rainy season. The high sensitivity of these variables was due to the large contribution of exposure via bathing (Figure 5) and the high variability of their associated media contamination data (Figure 1) and activity data (Figure 3). As indicated in Figure 3, the daily duration of bathing showed high variability, indicating that human behavior during bathing varies greatly among individuals. Such variability in human behavior was also found in previous studies (Dorevitch *et al.* 2011; Schets *et al.* 2011). In addition to the duration of bathing, pond bathing is a common practice in South Asia; however, no study has been conducted on the exposure factor of such bathing. The present study used an exposure factor during swimming (Dorevitch *et al.* 2011). Although their bathing behavior seems to be similar to swimming, the exposure factor directly reflecting pond bathing common in South Asia will improve the accuracy of exposure analysis in this setting.

CONCLUSIONS

This study successfully demonstrated that seasons and genders significantly impact the fecal exposure trends in children from a slum area. Of the five pathways targeted, pond bathing and drinking contributed a large part of the monthly exposure in the present study on a Bangladeshi slum. Significant seasonal differences were observed for bathing activities, higher in the rainy season (2.59×10^2 CFU/day for boys; 6.19×10^{-1} CFU/day for girls) than the dry season (1.69×10^2; 4.30×10^{-2}), and eating, higher in the dry season (3.71×10; 3.22×10) than the rainy season (1.50×10; 1.24×10); these differences were due to seasonal differences in children's activity and environmental media contamination. Significant gender differences were also observed in the bathing activities, higher in boys than in girls, due to differences in contamination levels of their preferred bathing locations. Pond bathing and drinking largely contributed to monthly exposure in various proportions for boys (33.3–42.7% for pond bathing; 42.2–52.9% for drinking) and that of drinking for girls (78.6–80.7%). This difference of major pathways in genders was caused by the difference of activity preference in genders. These findings suggest that seasonal and gender considerations will improve the assessment of fecal exposure through daily activities in settings where human behavior and environmental contamination change in seasons and genders.

This study has a few limitations. Certain input data were not obtained to separate the datasets by seasons and genders in the analysis. In addition, the exposure factors of certain activities were not obtained from the exact same activities. Although we examined the variability impact of exposure factors in the sensitivity analysis, exposure factors in a similar context would reduce the uncertainty of the assessment. Nevertheless, this study successfully demonstrated the distinct impact of seasons and genders on the fecal exposure of a slum setting, contributing to a more accurate exposure assessment reflecting local contexts.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Ahmed, T. & Sen, B. 2018 Conservative outlook, gender norms and female wellbeing: evidence from rural Bangladesh. *World Development* **111**, 41–58. https://doi.org/10.1016/j.worlddev.2018.06.017.

Aihara, Y. 2014 Handwashing and microbial contamination on the palms of preschool children in Kathmandu, Nepal. *Journal of International Health* **29** (2), 69–74. https://doi.org/10.11197/jaih.29.69.

Amin, N., Rahman, M., Raj, S., Ali, S., Green, J., Das, S. & Moe, C. L. 2019 Quantitative assessment of fecal contamination in multiple environmental sample types in urban communities in Dhaka, Bangladesh using SaniPath microbial approach. *PLoS ONE* **14** (12), 1–21. https://doi.org/10.1371/journal.pone.0221193.

Balk, D. 1997 Defying gender norms in rural Bangladesh: a social demographic analysis. **51** (2), 153–172. https://doi.org/10.1080/0032472031000149886

Bangladesh Bureau of Statistics 2014 *Bangladesh Population and Housing Census 2011*. Bangladesh Bureau of Statistics (BBS), Dhaka, Bangladesh.

Barro, N., Bello Abdoul, R., Savadogo, A., Ouattara Cheik Amadou, T., Ilboudo, A. & S, T. A. 2006 Hygienic status assessment of dish washing waters, utensils, hands and pieces of money from street food processing sites in Ouagadougou (Burkina Faso). *African Journal of Biotechnology* **5**, 1107–1112.

Bauza, V., Ocharo, R. M., Nguyen, T. H. & Guest, J. S. 2017 Soil ingestion is associated with child diarrhea in an urban slum of Nairobi, Kenya. *American Journal of Tropical Medicine and Hygiene* **96** (3), 569–575. https://doi.org/10.4269/ajtmh.16-0543.

Beamer, P., Key, M. E., Ferguson, A. C., Canales, R. A., Auyeung, W. & Leckie, J. O. 2008 Quantified activity pattern data from 6 to 27-month-old farmworker children for use in exposure assessment. *Environmental Research* **108** (2), 239–246. https://doi.org/10.1016/j.envres.2008.07.007.

Bhaskar, J., Usman, M., Smitha, S. & Bhat, G. K. 2004 Bacteriological profile of street foods in Mangalore. *Indian Journal of Medical Microbiology* **22** (3), 197.

Campos, L. C., Ross, P., Nasir, Z. A., Taylor, H. & Parkinson, J. 2015 Development and application of a methodology to assess sanitary risks in Maputo, Mozambique. *Environment and Urbanization* **27** (2), 371–388. https://doi.org/10.1177/0956247815595784.

Chowdhury, F. R., Shihab, Q., Ibrahim, U., Bari, S., Alam, J., Dunachie, S. J. & Patwary, I. 2018 The association between temperature, rainfall and humidity with common climate-sensitive infectious diseases in Bangladesh. *PLoS ONE* **13** (6), 1–17. https://doi.org/10.1371/journal.pone.0199579%0A

Dorevitch, S., Panthi, S., Huang, Y., Li, H., Michalek, A. M., Pratap, P. & Li, A. 2011 Water ingestion during water recreation. *Water Research* **45** (5), 2020–2028. https://doi.org/10.1016/j.watres.2010.12.006.

Ferdous, J. & Mallick, D. 2019 Norms, practices, and gendered vulnerabilities in the lower Teesta basin, Bangladesh. Environmental Development **31**, 88–96. https://doi.org/10.1016/j.envdev.2018.10.003.

Goddard, F. G. B., Pickering, A. J., Ercumen, A., Brown, J., Chang, H. H. & Clasen, T. 2020 Faecal contamination of the environment and child health: a systematic review and individual participant data meta-analysis. *The Lancet Planetary Health* **4** (9), e405–e415. https://doi.org/10.1016/S2542-5196(20)30195-9.

Haque, R., Parr, N. & Muhidin, S. 2020 Climate-related displacement, impoverishment and healthcare accessibility in mainland Bangladesh. *Asian Population Studies* **16** (2), 220–239. https://doi.org/10.1080/17441730.2020.1764187.

Harada, H., Fujimori, Y., Gomi, R., Ahsan, M. N., Fujii, S., Sakai, A. & Matsuda, T. 2018 Pathotyping of *Escherichia coli* isolated from community toilet wastewater and stored drinking water in a slum in Bangladesh. *Letters in Applied Microbiology* **66** (6), 542–548. https://doi.org/10.1111/lam.12878.

Japan Association of Drainage and Environment (JADE). 2016 Activity to Improve the Living Environment of Urban Slum Aiming at Resource Recycling in Khulna City of Bangladesh. Project Report (May 2012 to May 2015). JADE, Dhaka.

Julian, T. R., Vithanage, H. S. K., Chua, M. L., Kuroda, M., Pitol, A. K., Nguyen, P. H. L. & Harada, H. 2018 High time-resolution simulation of *E. coli* on hands reveals large variation in microbial exposures amongst Vietnamese farmers using human excreta for agriculture. *Science of the Total Environment* **635**, 120–131. https://doi.org/10.1016/j.scitotenv.2018.04.100.

Kato, T., Miura, T., Okabe, S. & Sano, D. 2013 Bayesian modeling of enteric virus density in wastewater using left-censored data. *Food and Environmental Virology* **5** (4), 185–193. https://doi.org/10.1007/s12560-013-9125-1.

Khatun, M. A., Rashid, M. B. & Hygen, H. O. 2016 *MET Report – Climate of Bangladesh*. No. 08/2016, pp. 1–158.

Kostyla, C., Bain, R., Cronk, R. & Bartram, J. 2015 Seasonal variation of fecal contamination in drinking water sources in developing countries: a systematic review. *Science of the Total Environment* **514**, 333–343. https://doi.org/10.1016/j.scitotenv.2015.01.018.

Kwiringira, J., Atekyereza, P., Niwagaba, C., Kabumbuli, R., Rwabukwali, C., Kulabako, R. & Günther, I. 2016 Seasonal variations and shared latrine cleaning practices in the slums of Kampala city, Uganda. *BMC Public Health* **16** (1), 361. https://doi.org/10.1186/s12889-016-3036-7.

Mohsenipour, M., Shahid, S., Ziarh, G. F. & Yaseen, Z. M. 2020 Changes in monsoon rainfall distribution of Bangladesh using quantile regression model. *Theoretical and Applied Climatology* **142** (3–4), 1329–1342. https://doi.org/10.1007/s00704-020-03387-x.

Nayebare, J. G., Owor, M. M., Kulabako, R., Campos, L. C., Fottrell, E. & Taylor, R. G. 2020 WASH conditions in a small town in Uganda: how safe are on-site facilities? *Journal of Water Sanitation and Hygiene for Development* **10** (1), 96–110. https://doi.org/10.2166/washdev.2019.070.

Osiemo, M. M., Ogendi, G. M. & M'Erimba, C. 2019 Microbial quality of drinking water and prevalence of water-related diseases in Marigat Urban Centre, Kenya. *Environmental Health Insights* **13**. https://doi.org/10.1177/1178630219836988

Pickering, A. J., Julian, T. R., Mamuya, S., Boehm, A. B. & Davis, J. 2011 Bacterial hand contamination among Tanzanian mothers varies temporally and following household activities. *Tropical Medicine & International Health: TM & IH* **16** (2), 233–239. https://doi.org/10.1111/j.1365-3156.2010.02677.x.

Pickering, A. J., Ercumen, A., Arnold, B. F., Kwong, L. H., Parvez, S. M., Alam, M. & Luby, S. P. 2018 Fecal indicator bacteria along multiple environmental transmission pathways (water, hands, food, soil, flies) and subsequent child diarrhea in rural Bangladesh. *Environmental Science and Technology* **52** (14), 7928–7936. https://doi.org/10.1021/acs.est.8b00928.

Pleil, J. D., Sobus, J. R., Stiegel, M. A., Hu, D., Oliver, K. D., Olenick, C. & Funk, W. E. 2014 Estimating common parameters of lognormally distributed environmental and biomonitoring data: harmonizing disparate statistics from publications. *Journal of Toxicology and Environmental Health – Part B: Critical Reviews* **17** (6), 341–368. https://doi.org/10.1080/10937404.2014.956854.

Prüss-Ustün, A., Wolf, J., Bartram, J., Clasen, T., Cumming, O., Freeman, M. C. & Johnston, R. 2019 Burden of disease from inadequate water, sanitation and hygiene for selected adverse health outcomes: an updated analysis with a focus on low- and middle-income countries. *International Journal of Hygiene and Environmental Health* **222** (5), 765–777. https://doi.org/10.1016/j.ijheh.2019.05.004.

Qadi, M., Kharraz, L., Shaheen, A., Amleh, A., Ibrahim, F., Abu Saleh, D. & Khayyat, R. 2019 Assessment of the hygiene-related risks of child illness at selected elementary schools in Nablus city: a cross-sectional survey. *The Lancet* **393**, S41. https://doi.org/10.1016/s0140-6736(19)30627-0.

Rahman, H. M. T., Mia, M. E., Ford, J. D., Robinson, B. E. & Hickey, G. M. 2018 Livelihood exposure to climatic stresses in the north-eastern floodplains of Bangladesh. *Land Use Policy* **79**, 199–214. https://doi.org/10.1016/j.landusepol.2018.08.015.

Raj, S. J., Wang, Y., Yakubu, H., Robb, K., Siesel, C., Green, J. & Moe, C. L. 2020 The SaniPath Exposure Assessment Tool: a quantitative approach for assessing exposure to fecal contamination through multiple pathways in low resource urban settlements. *PLoS ONE* **15** (6), 1–18. https://doi.org/10.1371/journal.pone.0234364.

R Core Team 2016 *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, Austria. Available from: https://www.R-project.org/.

Roseberry, A. M. & Burmaster, D. E. 1992 Lognormal distributions for water intake by children and adults. *Risk Analysis* **12** (1), 99–104.

Rufener, S., Mäusezahl, D., Mosler, H.-J. & Weingartner, R. 2010 Quality of drinking-water at source and point-of-consumption–drinking cup as a high potential recontamination risk: a field study in Bolivia. *Journal of Health, Population, and Nutrition* **28**(1), 34–41. https://doi.org/10.3329/JHPN.V28I1.4521.

Schets, F. M., Schijven, J. F. & de Roda Husman, A. M. 2011 Exposure assessment for swimmers in bathing waters and swimming pools. *Water Research* **45** (7), 2392–2400. https://doi.org/10.1016/j.watres.2011.01.025.

Šmilauer, P. & Lepš, J. 2014 Permutation tests and variation partitioning. In: *Multivariate Analysis of Ecological Data using CANOCO 5*, pp. 71–91. https://doi.org/10.1017/CBO9781139627061.006.

Taulo, S., Wetlesen, A., Abrahamsen, R. K., Narvhus, J. A. & Mkakosya, R. 2009 Quantification and variability of *Escherichia coli* and *Staphylococcus aureus* cross-contamination during serving and consumption of cooked thick porridge in Lungwena rural households, Malawi. *Food Control*. https://doi.org/10.1016/j.foodcont.2009.03.009

Troeger, C., Blacker, B. F., Khalil, I. A., Rao, P. C., Cao, S., Zimsen, S. R. & Reiner, R. C. 2018 Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Infectious Diseases* **18** (11), 1211–1228. https://doi.org/10.1016/S1473-3099(18)30362-1.

U.S. Environmental Protection Agency (EPA) 2005 *Guidance on Selecting Age Groups for Monitoring and Assessing Childhood Exposures to Environmental Contaminants.* p. 50.

U.S. Environmental Protection Agency 2011 *Exposure Factors Handbook: 2011 Edition.* EPA/600/R-09/052F. U.S. Environmental Protection Agency, Washington, DC, pp. 1–1466.

Van Geen, A., Ahmed, K. M., Akita, Y., Alam, M. J., Culligan, P. J., Emch, M. & Yunus, M. 2011 Fecal contamination of shallow tubewells in Bangladesh inversely related to arsenic. *Environmental Science and Technology* **45** (4), 1199–1205. https://doi.org/10.1021/es103192b.

Wang, Y., Moe, C. L. & Teunis, P. F. M. 2018 Children are exposed to fecal contamination via multiple interconnected pathways: a network model for exposure assessment. *Risk Analysis* **38** (11), 2478–2496. https://doi.org/10.1111/risa.13146.

WHO 2016 *Quantitative Microbial Risk Assessment: Application for Water Safety Management.* WHO Press, p. 187. Available from: http://www.who.

WHO and UNICEF 2018 Core questions on water, sanitation and hygiene for household surveys – 2018 update. *JMP for Water Supply Sanitation and Hygiene*, pp. 1–24. Available from: https://washdata.org.

Wilson, A. M., Verhougstraete, M. P., Beamer, P. I., King, M. F., Reynolds, K. A. & Gerba, C. P. 2020 Frequency of hand-to-head, -mouth, -eyes, and -nose contacts for adults and children during eating and non-eating macro-activities. *Journal of Exposure Science and Environmental Epidemiology*. https://doi.org/10.1038/s41370-020-0249-8.

Wymer, L. J. & Wade, T. J. 2007 The lognormal distribution and use of the geometric mean and the arithmetic mean in recreational water quality measurement. In: *Statistical Framework for Recreational Water Quality Criteria and Monitoring* (Wymer, L. J., ed.). John Wiley & Sons, Chichester, chap. 6.

Xue, J., Zartarian, V. G., Özkaynak, H., Dang, W., Glen, G., Smith, L. & Stallings, C. 2006 A probabilistic arsenic exposure assessment for children who contact chromated copper arsenate (CCA)-treated playsets and decks, part 2: sensitivity and uncertainty analyses. *Risk Analysis* **26** (2), 533–541. https://doi.org/10.1111/j.1539-6924.2006.00748.x.