The Landscape of Enteric Pathogen Exposure for Children during Play in Public Domains of Low-Income, Kisumu, Kenya.

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Abstract

Recent studies have highlighted tremendous diversity in the microbial etiology of enteric infection in young children in low-income, disease-endemic countries. Yet, little is known about which conditions are most important in the exposure and infection of children. This study examined how the dose of six types of enteric pathogens, and the probability of exposure to one or more enteric pathogens, was influenced by the type and frequency of child play behaviors that result in contact with soil and surface water at public play areas in Kisumu, Kenya, and localized- versus neighborhood-level of exposure. A multivariate random effects tobit model was used to obtain the posterior distribution of pathogen concentrations and their dependencies in soil and surface water and exposure doses were estimated using contact fate parameters from previous studies. Exposure doses and probability of multi-pathogen ingestion increased with increased frequency of environmental contact, especially for surface water versus soil, and exposure to multiple sites in the neighborhood. Multi-pathogen exposure was common, with a >60% probability of ingesting all six pathogens after just a few contacts with surface water. This evidence suggests that contact with surface water and exposure to soil from multiple neighborhood locations are major determinants of children in Kisumu ingesting high doses of multiple types of pathogens.

INTRODUCTION

Recent studies have highlighted tremendous diversity in the microbial etiology of enteric infection in children less than five years of age (<5) in low income, disease endemic countries.[1-8] This demonstrates that young children are exposed to a variety of pathogens from unhygienic living conditions on a regular basis. Little is known about the rate with which children are exposed to and acquire new infections over time. Based upon the fact that children experience an average of 4 to 8 diarrheal episodes per year between birth and 2 years of age,[9] it is likely that children are exposed to a variety of pathogen types beginning in the first year of life. Diarrheal episodes may underestimate how often children acquire new infections, given that many infections are asymptomatic and go undetected without extensive diagnostic profiling.[10] Diarrhea rates may even further underestimate how often children are exposed to pathogens, but remain uninfected due to insufficient exposure dose, lack of pathogen viability, host acquired immunity, or other mediating conditions. Adding to this complexity, co-infection of individual children by two or more types of pathogens – regardless of symptomatic status – is common.[1-2] Co-infection, even in the absence of diarrheal symptoms, elevates the risk of environmental enteropathy, malnutrition, and subsequent co-infection by a new pathogen, perpetuating the cycle of disease.[3] Understanding which exposure pathways increase the frequency of multi-pathogen exposure of children could improve the prioritization of interventions that reduce early childhood enteric disease incidence.

The decades-old F diagram conceptualized environmental exposure pathways according to the properties of materials (drinking water, food, soil, etc.) that could be contaminated by feces and enter the mouth of humans.[11] However, the overall risk and rate of exposure to enteric pathogens over time may be fundamentally different across exposure pathways; for example eating household soil where animals defecate may be much more hazardous than repeat ingestion of household drinking water.[12-14] The importance of place, i.e. household versus public domains, is also not represented in the F diagram.

59 Eating soil during play at an open defecation site may be more hazardous than eating household soil. If
60 this is the case, the F diagram needs to be re-imagined so that pathways resulting in elevated exposure
61 and infection risks are given greater weight when prioritizing interventions.

62 Currently data on the rate at which children experience multi-pathogen exposure and infection,
63 and how these rates vary across exposure pathways is lacking. Pathway comparisons to date have relied
64 on fecal indicator bacteria concentrations,[13, 15-18] or pathogen-specific risks,[19-21] neither of which provide
65 information about cumulative enteric pathogen exposure over space and time. High detection
66 frequencies of different types of pathogens in households of India and Tanzania and public play areas in
67 Kenya suggests multi-pathogen exposure is plausible.[22-25] However, only one report to our knowledge
68 attempted to estimate exposure to multiple types of pathogens.[26] The modeling approach summed the
69 individual probabilities of exposure to each type of pathogen from South African surface waters, but
70 assumed pathogens occurred independently of one another across sampling locations and that the risk
71 of exposure to each pathogen was equal. Our group has shown that pathogen diversity at child play
72 areas in Kisumu is a continuum from areas with no contamination to areas contaminated by multiple
73 types of pathogens.[24] This suggests unsafe play behaviors by children in Kisumu could result in
74 simultaneous ingestion of multiple pathogens at some public sites. Simply adding pathogen-specific
75 probabilities of exposure fails to account for interdependence in pathogen contamination across
76 exposure pathways or exposure locations. It also does not capture how spatial variability of young
77 children's play in neighborhoods could influence the dose and diversity of pathogen exposure. Many
78 children play unattended while others have self- or guardian- driven limitations that restrict distance
79 away from the household and acceptable areas for play.[25] Children who play in a constrained spatial
80 area (i.e. near their household) may experience different risks for pathogen exposure than children who
81 roam across a larger spatial area and encounter a variety of unsafe environmental conditions
82 throughout the course of a day.

New exposure assessment approaches that incorporate information about spatial variability in pathogen co-detection in the environment are needed to accurately understand the relative importance of different environmental exposure pathways on enteric infection among children in disease endemic settings. Such approaches can then be used to examine where and how exposures pathways influence infection incidence patterns. The first objective of this study was to measure how increased frequency and volume of contact with soil and surface water by children <5 yrs at public play areas in Kisumu influences the ingestion dose of DNA or RNA from different types of enteric pathogens, and the probability of exposure to one or more enteric pathogens. Second, we examined how increased spatial range of play for children influences dose and probability of multi-pathogen exposure.

METHODS

Study Design.

Observational assessment and environmental microbiology data on public sites in Kisumu, Kenya has been described previously.[24] Of all public sites where children <5 yrs were observed in Kisumu, 93% of these sites were classified as peri-domestic areas with mostly permeable or unpaved surfaces. Further, 54% of peri-domestic sites (n=116) were observed to have at least one child <5 yrs playing in the vicinity, compared to 8% of non-peri-domestic sites (n=50) observed to have at least one child playing in the area. Therefore, only environmental data from public sites classified as peri-domestic areas were included in the model, totaling 125 soil samples and 34 surface water samples, where 30 soil samples and 3 surface water samples were randomly selected resamples.[24]

Pathogens which were detected in less than 5% of the soil and surface water samples were excluded from the model to ensure sufficient knowledge about pathogen distributions and to obtain numerical stability in the statistical estimation algorithms. Of the 19 pathogens tested during environmental sampling, concentration data for 6 pathogens (Table S1) were eligible for inclusion in the model: *Cryptosporidium spp., Giardia,* human adenovirus 40/41*,* Enteropathogenic *E. coli* (EPEC)*,*

Enterotoxigenic *E. coli* (ETEC), and Enteroaggregative *E. coli* (EAEC).[24] Although the dataset was restricted to 6 of the 19 pathogens measured, the number of soil and surface water samples with at least one positive detect (138/159 samples) did not change, indicating that the 6 pathogens selected reasonably represent the overall contamination level of the environment. Differentiating gene markers for pathogenic *E.coli* were collapsed into categories of EPEC (*bfpA* and/or *eae)*, ETEC (*EST* and/or *elt*), and EAEC (*aiicA* and/or *aatA*). If there was a positive detect for more than one gene marker, and concentrations (C_p) varied between gene markers, concentrations of ETEC-*estA*, EPEC-*bfpA*, and EAEC-*aatA* were prioritized over concentrations of ETEC-*eltB*, EPEC-eaeA, and EAEC-aaiC, respectively, based on etiological importance in pediatric diarrheal disease.[1-2]

Statistical Analyses.

The overarching goal of the statistical analyses was to estimate the dose distributions of each pathogen type in soil or surface water by contact type, frequency of contacts, and spatial range of exposure. A Bayesian framework was employed to obtain the posterior distribution of pathogen doses for a certain number of contacts, denoted as $D(k)$ for k contact events, i.e., the distribution of $D(k)$ implied by information provided by both our data and previous studies.[14, 16, 27-31] The posterior distribution yields point estimates and credible intervals for the parameters of the pathogen concentration distribution, denoted as θ, for soil and water samples.

There are two parts to our modeling framework. The first part uses environmental microbiology data (Table S1) to estimate the distribution of each pathogen concentration in soil or surface water.[24] The second part combines the concentration distribution of part one with contact fate parameters provided from previous studies to estimate the dose distribution by contact type and frequency. The posterior distribution of interest, namely that of $D(k)$ and θ given the observed data and information from previous studies, can be decomposed to clearly reveal these two components of the statistical model:

$$\Pr(D(k), \theta | data, previous\ studies) = \Pr(D(k)|\theta, \text{previous studies}) \cdot \Pr(\theta|\text{data})$$

In implementation, we estimated this posterior distribution via a Monte Carlo approach on the joint posterior distribution augmented with the pathogen concentration corresponding to the k events. That is, we may first obtain a sample of θ from the marginal posterior distribution given the data, then draw k pathogen concentrations for the current value of θ, and finally, given those concentrations and information obtained from previous studies on exposure pathways, draw $D(k)$.

Pathogen Concentration Distribution Parameters.

Several challenges arose in estimating the parameters θ for the pathogen concentration distributions. First, there was left censoring caused by methodologically-constrained lower limits of detection (Table S1). Second, there were two important sources of dependency in the data—that which occurs due to the correlations between the different pathogens, and that which occurs due to resampling near the same site.

To handle data challenges, we fit a multivariate random effects tobit model to the log transformed concentration data. The first source of dependency in the data was accounted for by modeling all pathogens jointly rather than running many univariate analyses. It was also important that we not neglect to account for the latter type of dependency described above, as the spatial patterns of young children playing in neighborhoods could influence the dose and diversity of pathogen exposure in public areas. Thus, the proposed random effects included in our multivariate tobit model account for this spatial dependence. See the Supplemental Information for details on this statistical model.

For each environmental sample type, samples of θ were obtained from the posterior distribution using a Gibbs sampler. From these samples, posterior draws of pathogen concentrations of k new events were drawn from a multivariate normal distribution parameterized by the draws of θ. See the Supplemental Information for details on the Gibbs sampling algorithm.

Exposure Pathways.

During rapid observation of public sites in Kisumu, children <5 yrs were observed walking or running and playing or crawling on the ground at 41% and 17% of 116 peri-domestic public sites, respectively. Children played in puddles of surface water or mud at two sites. Young children typically have high rates of contact with soil and objects, have been observed to touch surface water, and frequently place their hands in the mouth with no handwashing in between,[25] resulting in *indirect* ingestion of trace amounts of soil and surface water. Just one act of geophagia (*direct* ingestion of handfuls of soil) was observed in Kisumu, although these were spot observations of public sites that lasted less than 15 minutes. Several other behavioral studies have reported geophagia among young children, including in public areas.[12, 14, 25, 32] Although no children were observed to drink directly from a surface water source during rapid observations in Kisumu, Kenya, young children were observed drinking directly from a surface water source at public sites in a peri-urban community in Haiti.[25] Even infrequent, rare behaviors such as geophagia, or consumption of surface water, are important pathways to gastrointestinal (GI) pathogen exposure during play in public sites.[12, 14, 33]

A theoretical model was developed to estimate and compare the dose and diversity of enteric pathogens ingested by young children via indirect and direct exposure to soil and surface water at public play areas. The contact frequency was held at a constant rate, ranging from a minimum of 1 to a maximum of 10 contacts, for pattern comparison purposes, so behaviors in this model are not weighted to account for the likelihood of engaging in the behavior and the rate of contact given a child plays in a public area for a specified timespan.[25] Therefore, the results are not cumulative estimates of actual child exposure, but represent possible exposures given a range of possible conditions. To obtain a posterior sample of the final dose for each set of conditions, the k concentrations drawn previously were multiplied by fate parameters, each drawn from a random distribution to account for the inherent variability in such occurrences, and then the k doses ranging from 1 to 10 were summed. The spatial

assumption determined whether or not the k contacts were correlated. The formulas used to estimate the dose distribution from indirect and direct contact with soil (A) and surface water (B) are:

A. Soil

$$\text{Soil-Hand-Mouth Dose}_p = C_{Sp} \times TE_S \times SA_{Hi} \times {}^*F_{HO} \times \left(\dagger \frac{{}^*F_{HM}}{F_{HO}}\right) \times TE_{HM}$$

$$\text{Geophagia Dose}_p = C_{Sp} \times V_S$$

B. Surface Water

$$\text{Water-Hand-Mouth Dose}_p = C_{Wp} \times TE_W \times SA_{Hi} \times {}^*F_{HO} \times \left(\dagger \frac{{}^*F_{HM}}{F_{HO}}\right) \times TE_{HM}$$

$$\text{Drink Surface Water Dose}_p = C_{Wp} \times V_W$$

Where * means truncated with a lower bound=0 and upper bound=1, † means the surface area of hand-to-mouth contact cannot exceed the surface area that was contaminated during hand-to-object contact, and the fraction truncated at 1. The p^{th} pathogen is denoted by a subscript of p.

Fate parameters obtained from the extant literature to estimate exposure to pathogens through indirect contact include: the transfer efficiency of the environmental fomite to the hand, the surface area of the child's hand contacting the environmental fomite, the surface area of the hand mouthed by the child, and the transfer efficiency of environmental residual from hand-to-mouth (Table 2). Total hand surface area (cm^2) used in this model was based on estimated surface area parameters for children between the ages of six months to less than six years.[27] Standard deviation for total hand surface area (cm^2) per age category (6 to 11 months, 12 to 23 months, 24 to 35 months, and 36 to 72 months) was calculated by dividing the difference of the EPA-reported mean and 95th percentile for hand surface area by the 95th quantile of a standard normal distribution (1.645).[27] Each age category was equally represented during simulation by sampling the probability of obtaining a random child within each of the unequal month spans and their respective mean and standard deviation (SA_{Hi}). The distribution for the fraction of the child's hand involved in hand-to-object contact (F_{HO}) and hand-to-mouth contact (F_{HM}) was calculated by minimizing the squared differences between theoretical and empirical quantiles.[30]

Transfer efficiency of environmental residue from hand-to-mouth (TE_{HM}) was estimated with a single point estimate due to the lack of literature to infer a distribution for all pathogens used in this analysis.[31] Our exposure model assumes that the hand region that contacted the object is the same region that contacted the mouth. This assumption is supported by the finding that regardless of the type of interaction, hand contact predominantly involves the fingers.[30] When summing across estimated indirect doses, hand size was held constant, while soil adherence and hand area that contacted the object and then mouth varied between summed contact events. To estimate direct ingestion of soil or surface water, the volume of respective substance placed in the mouth during a geophagia (V_S) or drinking occurrence (V_W) was estimated with a single parameter because of the lack of literature to describe the distribution of direct ingestion occurrences (Table 1).[14, 16, 33]

Table 1. Description of variables and parameters used to estimate distribution of indirect and direct doses.

Description	Symbol	Parameter	Source
Pathogen concentration	C		
Soil/gram	C_{Sp}*	See Table S1	This study
Surface water/mL	C_{Wp}	See Table S1	This study
Transfer efficiency from object	TE		
Soil adherence	TE_S	Lognormal (0.52,0.9) mg/cm^2 **Converted C_{Sp} to mg*	Finley et al. (1994) [29]
Water film thickness	TE_W	Uniform (0.00234, 0.00499) cm	EPA Exposure (1987) [28]
Surface area of hand	SA_{Hi}		
6 to <12 months	SA_{H1}	Normal (240, 18.2) cm^2	EPA Exposure (2011) [27]
1 to <2 years	SA_{H2}	Normal (300, 30.4) cm^2	EPA Exposure (2011) [27]
2 to <3 years	SA_{H3}	Normal (280, 30.4) cm^2	EPA Exposure (2011) [27]
3 to <6 years	SA_{H4}	Normal (370, 54.7) cm^2	EPA Exposure (2011) [27]
Fraction of hand	F_H		
Contacting object	F_{HO}	Normal (0.215, 0.111) %	Auyueng et al. (2008) [30]
Contacting mouth	F_{HM}	Normal (0.18, 0.076) %	Auyueng et al. (2008) [30]

Transfer efficiency from hand	TE_H		
Hand-to-mouth	TE_{HM}	0.33 (%)	Rusin et al. (2002) [31]
Volume ingested during direct ingestion	V		
Geophagia	V_S	1.25 grams	Ngure et al. 2013 [14]
Drinking surface water	V_W	5 mL	Labite et al. 2010 [16]

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210 **RESULTS**

211 **Correlation in Pathogen Concentrations.**

212 Estimated pathogen concentration distributions for soil and surface water (θ) can be found in
213 Table S2. Pathogens concentrations were all positively correlated in surface water, but many were not
214 positively correlated in soil (Figure 1). The 95% credible intervals (CI) for the correlations revealed that
215 only four pathogen comparisons in surface water were significantly correlated at the site- and
216 neighborhood-level: adenovirus 40/41 vs. EPEC (site CI: 0.05, 0.89; neighborhood CI: 0.17, 0.77),
217 adenovirus 40/41 vs. EAEC (site CI: 0.03, 0.87; neighborhood CI: 0.15, 0.73), ETEC vs. EPEC (site CI: 0.1,
218 0.86; neighborhood 0.20, 0.74), and EPEC vs. EAEC (site CI: 0.15, 0.88; neighborhood CI: 0.20, 0.75)
219 (Figure S1). In soil, significant neighborhood-level negative correlation was observed for
220 *Cryptosporidium spp.* vs. adenovirus 40/41 (CI: -0.56, -0.14), and positive correlation for EAEC vs.
221 adenovirus 40/41 (CI: 0.06, 0.61), and EAEC vs. ETEC (CI: 0.3, 0.76) (Figure S1). Overall, the strongest
222 pathogen correlations in soil and surface water were observed among human adenovirus 40/41 and
223 pathogenic E. *coli* and among the E. *coli* themselves.

Figure 1. Site- and neighborhood-level correlation in concentration of enteric viruses, bacteria, and protozoans in soil and surface water in Kisumu, Kenya. Negative and positive correlation shown in the lower left quadrants of each grid are reflected in orange-red and blue circles, respectively, in the upper right quadrants of each grid. The highest rho indicated by the darkest color and narrowest shapes.

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Pathogen Exposure Doses by Behavioral Pathway and Spatial Range.

Pathogen doses from soil contact (Figure 2) were always lower than doses from surface water contact (Figure 3). All surface water contact resulted in a mean ingestion of at least one pathogen, with the exception of Giardia for one water-hand-mouth contact. If frequency of contact with soil or surface water is held constant, direct contact always resulted in higher pathogen doses compared to indirect contact (Figure 2C/2D vs. Figure 2A/2B; Figure 3C/3D vs. Figure 3A/3B). However, if indirect contact occurred more often than direct contact, then doses resulting from indirect contact could exceed doses by direct contact. For example, if a child exhibited ten soil-hand-mouth contacts and one geophagia contact at site-level play, the EAEC dose for soil-hand-mouth (~80 bacteria, Figure 2A, solid box) would exceed the EAEC dose for geophagia (~1 bacteria, Figure 2C, dashed box). Overall, pathogen doses from contact with soil or surface water increased as spatial scale expanded from site to neighborhood-level child exposure (Figure 2A/2C vs. Figure 2B/2D; Figure 3A/3C vs. Figure 3B/3D), but the magnitude of change depended upon the pathogen type.

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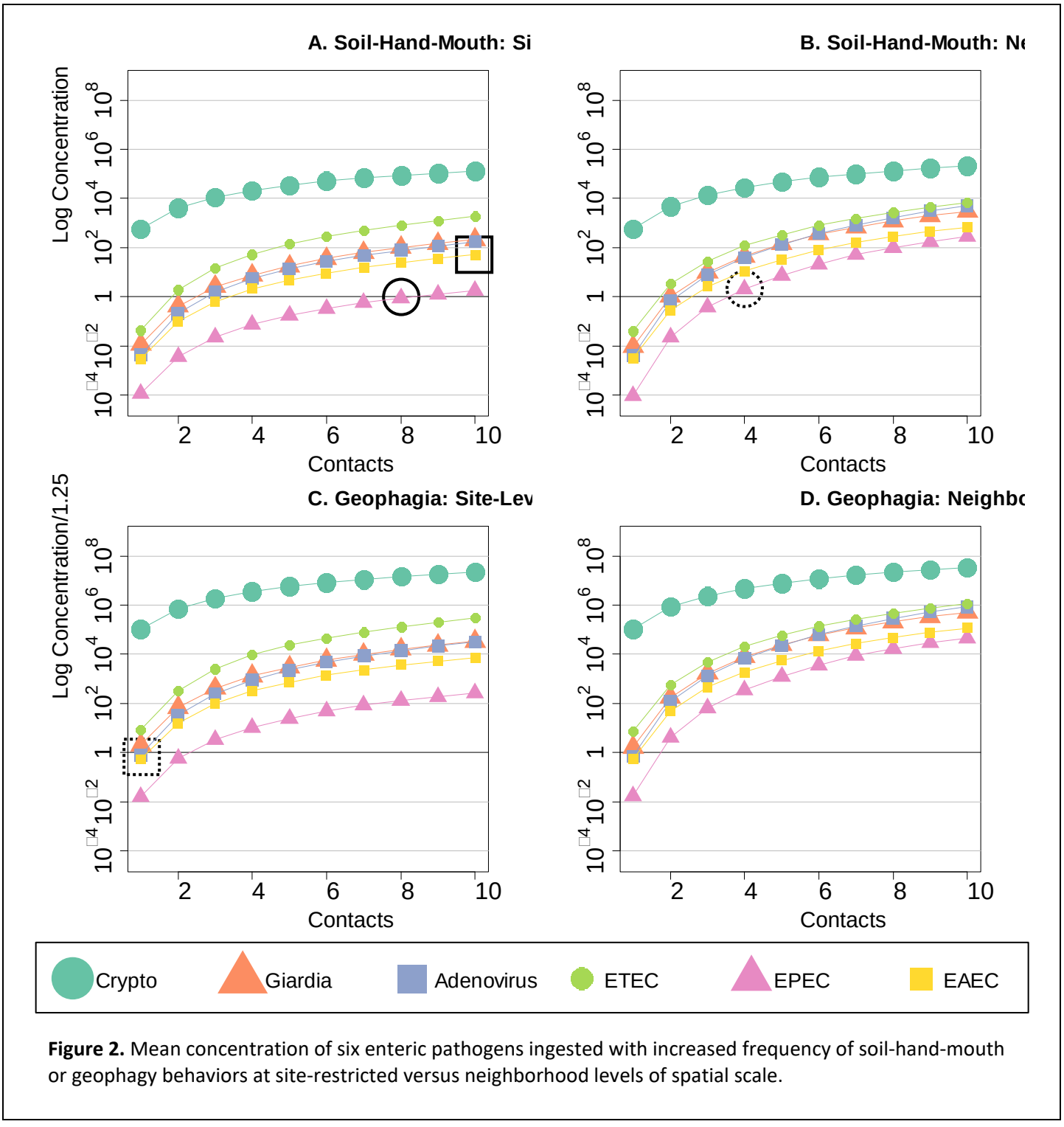

Figure 2. Mean concentration of six enteric pathogens ingested with increased frequency of soil-hand-mouth or geophagy behaviors at site-restricted versus neighborhood levels of spatial scale.

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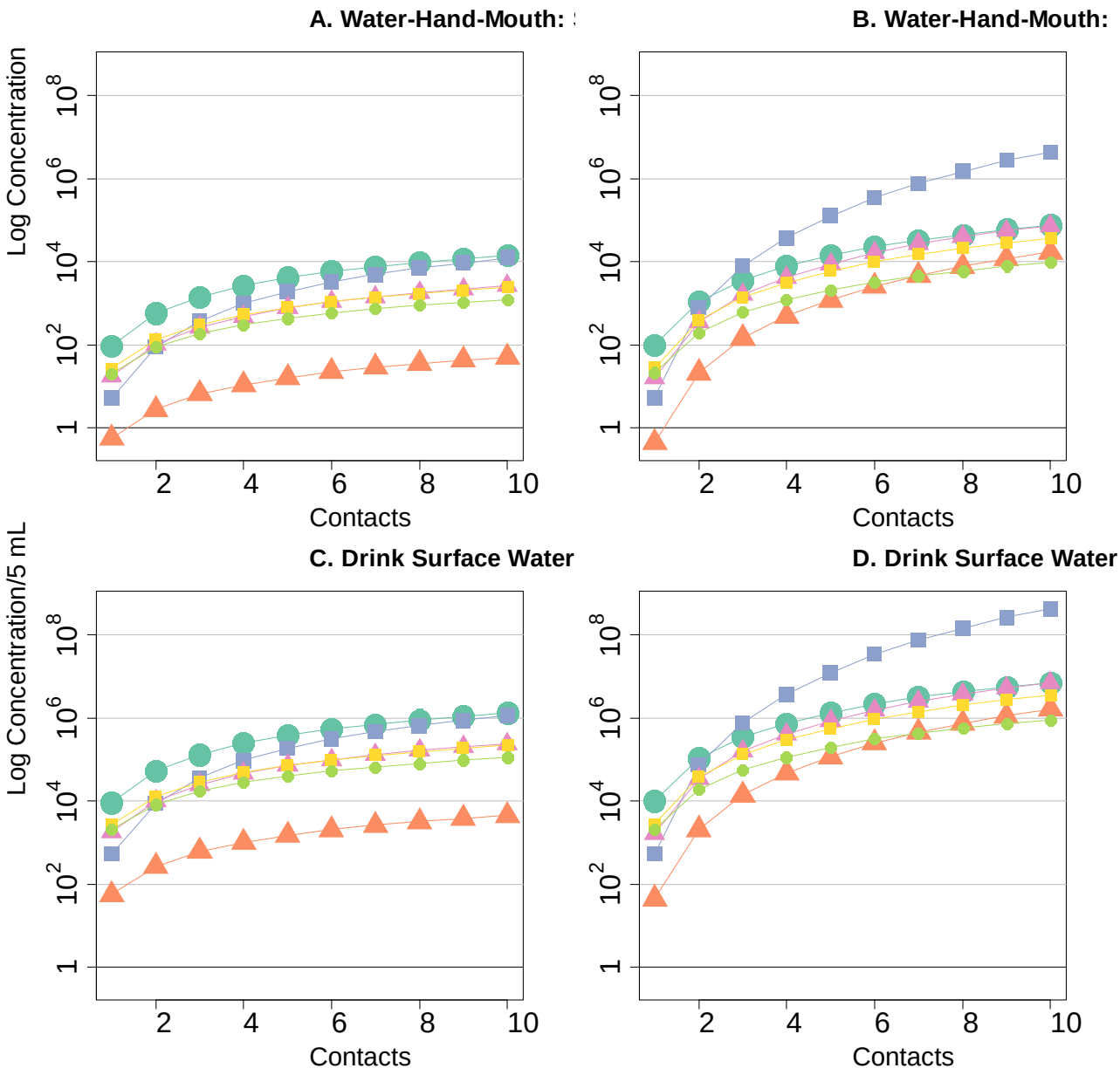

Figure 3. Mean concentration of six enteric pathogens ingested with increased frequency of hand-to-mouth or surface water drinking behaviors at site-restricted versus neighborhood levels of spatial scale.

Pathogen-Specific Exposure Dose Patterns.

Pathogen-specific dose distributions from indirect and direct contact with soil and surface water at site- and neighborhood-level are reported in Figures S2-S7. The mean dose $D(k)$ of pathogens ingested during any contact with soil were greatest for *Cryptosporidium spp.* (Figure 2). However, *Cryptosporidium spp.* dose did not considerably increase with increased behavior frequency or increased spatial scale of play from site- to neighborhood-level. The lowest pathogen dose ingested during soil contact was EPEC—yet the dose of EPEC substantially increased for neighborhood vs. site-level play. For example, it required two times more soil-hand-mouth contacts to ingest the same mean dose of EPEC at site-level play (~8 contacts, Figure 2A, solid circle) as neighborhood-level play (~4 contacts, Figure 2B, dashed circle). Most noticeably, the dose of human adenovirus 40/41 from surface water contact exponentially increased as spatial scale expanded from site to neighborhood play and surpassed all other pathogen doses at neighborhood-level exposure.

Exposure to Diverse Pathogen Types.

Figure 4 illustrates the probability of ingesting one or more pathogens for 1 to 10 indirect or direct contact(s) with soil or surface water during site-restricted or neighborhood play, where a successful ingestion is defined as 1 or more pathogen DNA. Across all behaviors, the probability of ingesting more than one pathogen type intensified as spatial scale expanded from site to neighborhood play. For example, the probability of ingesting six pathogens from two water-hand-mouth contacts during site-restricted play (44%, Figure 4E, solid box) increased by about 16% if the child exhibited the same behavior and frequency during neighborhood-level play (60%, Figure 4F, dashed box). Soil-hand-mouth contact resulted in the lowest risk of ingesting diverse pathogen types compared to all other behaviors practiced at the same frequency. This is especially evident for soil-hand-mouth contact during site-restricted play where the probability of ingesting all 6 pathogens did not exceed 35% for 10 contacts. Any contact with surface water posed high risk for ingestion of diverse pathogens and is

demonstrated by an exceedance of 90% probability of ingesting 6 pathogens during ≥5 water-hand-mouth contacts and ≥3 drinking water contacts during neighborhood-level play.

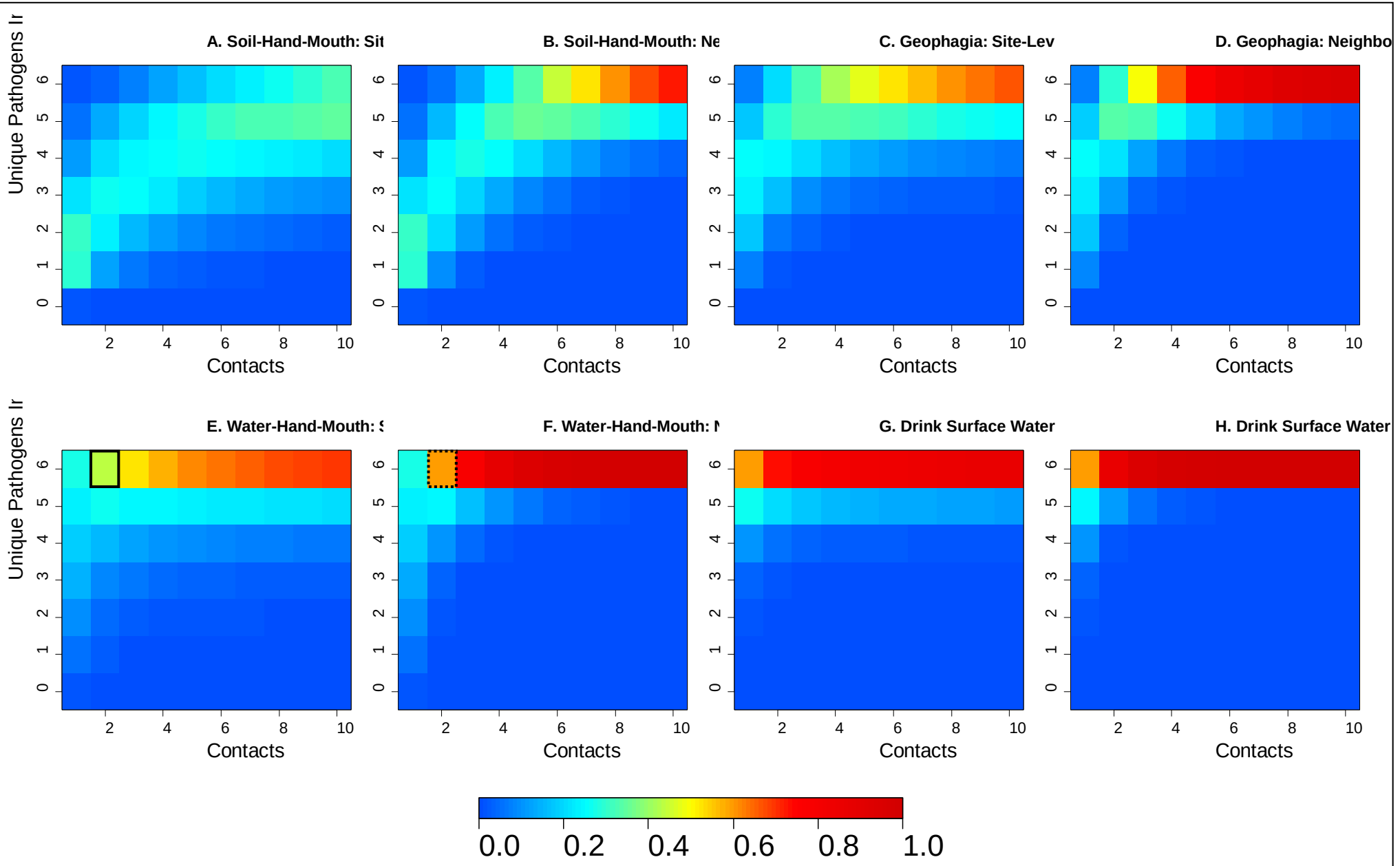

Figure 4. Probability of ingesting one or more enteric pathogens with increased frequency of exposure contact with soil or surface water at site-restricted and neighborhood levels of scale.

DISCUSSION

Children <5 yrs in low income neighborhoods of Kisumu, Kenya are exposed during play in residential public areas to soil and surface water contaminated by human and animal feces and a diverse range of enteric pathogens.[24] This exposure assessment study expands what is known about pathogen-specific doses and diversity in enteric pathogen types ingested by young children as a result of contact with soil or surface water from peri-domestic public areas. Our evidence shows that when holding frequency of behaviors constant, dose and probability of ingesting multiple enteric pathogens are greater when a child ingests: (1) surface water versus soil, (2) greater volumes of soil (geophagy) or surface water (small, incidental mouthfuls) versus hand-to-mouth contact, and (3) soil or surface water from multiple neighborhood locations versus just one spatially-restricted site.

In reality, the rate of each of these behaviors during child play are not similar, and there are currently no published studies describing how much time children in low income settings spend in public areas or how far they range to inform estimates of exposure risk. In our studies of child play in public areas in Haiti, geophagy was 6 times more common than drinking surface water (0.9/hr vs 0.15/hr), and hand-to-mouth was roughly 9 (child) to 20 (infant) times more common than geophagy.[25] We kept contact frequency at a constant rate to examine relative patterns of dose and diversity, but the importance of different behaviors in child exposure may be larger or smaller once adjusted for actual behavioral rates. If we assume that child behavior frequencies are generalizable across geographic contexts (e.g. children in Kenya behave like children in Haiti), then child exposure to low doses (10^0-10^2) of at least one type of pathogen from short durations of play outside their home is certain, due to the pervasiveness of pathogen contamination in these neighborhoods and frequent hand-to-soil and hand-to-mouth contacts.[25] Exposure doses and risk of multi-pathogen exposure escalates with increased movement of the child around the neighborhood or with increased contact with environmental fomites. Multi-pathogen exposure was common for even the lowest risk contact; for example the summed

probabilities for exposure to two or more pathogens for one soil-hand-mouth contact during site-level play was 69%. Yet, multi-pathogen exposure to all six pathogens rapidly reached saturation at only a few contacts with surface water, whereas the probability of total multi-pathogen exposure for contact with soil did not reach saturation as of 10 contact events.

There were some pathogen-specific differences in exposure dose concentration curves. *Cryptosporidium spp*. was the most common pathogen in soil, at relatively stable concentrations across the neighborhood, which led to higher exposure doses. Notably, the 18S subunit gene indicator used in this study to detect Cryptosporidium detects some species not common isolates associated with child infection. Sub-screening of a handful of samples did not detect *C. parvum* or *C. hominus* specific sequences. Thus, we suspect that the majority of exposure to Cryptosporidium in this study would not pose a risk to children. Pathogenic *E. coli* were also quite common. Similar exposure dose curves were observed for all pathogens in surface water, albeit at higher dose concentrations than in soil, except for human adenovirus, which demonstrated spatial dependencies. Human adenovirus concentrations were highly varied between sites, compared to other pathogens, which indicates that child exposure to different public sites was an important determinant of greater doses.

There are several key limitations of this study. This study relied upon qRT-PCR estimated concentrations of enteric viruses, bacteria, and protozoan pathogens to estimate exposure doses. While use of a multi-pathogen qRT-PCR process reduced methodological variability in quality of concentration estimates, it does not distinguish between viable and non-viable organisms in the environment. So, pathogen exposure doses presented in this study may overestimate the number of infectious pathogens children ingest through contact with soil and surface water. There is a lack of information on how well qRT-PCR correlates with other approaches for quantifying pathogen environmental exposure, and with child infection outcomes in settings like Kisumu. However, PCR detection of microbial source tracking (MST) markers in Indian households was associated with diarrhea in one study.[22] Like other culture-

based indicators, MST markers likely occur in the environment more frequently than infectious pathogens, and it is unclear whether MST presence is a reliable estimate of pathogen dose. Validating health outcomes associated with child exposure doses in Kisumu public areas was outside the scope of this study but is critical to understanding the importance of public exposure pathways in pediatric enteric infections. More information on dose-response in children in low income settings is needed that accounts for potential interdependencies in the presence of pathogens.

The findings of this study provide plausible explanations for why household WASH interventions often have minimal impact on child diarrhea,[34-36] and why global diarrhea disease burden remains high in spite of drastic improvements in basic water and sanitation access in recent decades.[37] Child play outside the household is far more common than appreciated and is likely to continue as long as families live in crowded, unsanitary conditions. Children playing unaccompanied or being cared for by older siblings may contribute to hazardous scenarios where children play in neighborhood areas and contact objects that are unsafe or unsanitary.[25] Household WASH interventions do not typically address the topic of where children play, and do not install barriers between children and soil and water contaminated by the feces of one's neighbors and domestic animals. Thus, while household WASH improvements can reduce child exposure and infection from some pathways, they may not sufficiently reduce exposure across all pathways to observe differences in diarrhea rates.

The evidence demonstrating that children can be exposed to multiple pathogens from play in their neighborhoods suggests another interesting hypothesis – that exposure in public play areas could plausibly result in simultaneous infection by several pathogens transmitted by the same pathway. Co-infection is often thought of as an additive set of events where infection and exposure are considered independent events, i.e. a child has a pre-existing infection and then ingests another type of pathogen through environmental exposure. Child infections by two different pathogens may not be time-independent events in settings where children play in unsanitary environments. Confirmation of these

hypotheses would have far reaching implications for understanding enteric disease transmission and prioritization of interventions. In conclusion, future WASH interventions need to target the conditions that result in children playing in heavily contaminated public areas around the household. While restricting young children to playing in household areas seems impractical, and even unethical from a social standpoint, interventions could seek to eliminate unattended or sibling-attended child play, educate all relevant child caregivers about safe child play environments, and seek to create human and animal defecation-free spaces in residential areas.

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