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Environmental transmission of diarrheal pathogens in low and middle income countries

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Every year, more than half a million children die due to diarrheal diseases. Recent studies have identified the most important etiologies of diarrheal disease are enterotoxigenic and enteropathogenic *E. coli*, *Shigella* spp., rotavirus, norovirus and *Cryptosporidium* spp. These etiologies are unsurprisingly characterized by a combination of high shedding, high infectivity, and transmissibility through multiple environmental reservoirs. The relative importance of the transmission routes is likely site-specific. So the impact of interventions, which typically target only one or two environmental reservoirs, is likely also site-specific. The factors influencing the transmission routes most important for diarrheal disease are complex, including – at a minimum – etiology of endemic disease; and water, sanitation, and hygiene infrastructure and practices. The site-specific nature – and complexity of transmission – helps explain the observed variation in impacts of water, sanitation, and hygiene interventions. It may also render efforts to estimate or quantify global means for interventions' impacts irrelevant. The theme of this *Perspective* is that greater reductions in diarrheal disease transmission in LMICs can be achieved by designing interventions to interrupt the most important environmental transmission pathways. Intervention choice should be informed by site-specific conditions, most notably: diarrheal etiology and existing water, sanitation, and hygiene infrastructure and practices. The theme is discussed through the lens of the characteristics of the most important diarrheal diseases (shedding, infectivity, growth, and persistence) and the general characteristics of environmental reservoirs (exposure pathways and fecal contamination). The discussion highlights when interventions – and combinations of interventions – will be most effective at reducing diarrheal disease burden.

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Environmental impact

Globally, more than half a million children die every year from diarrheal diseases. Recent studies have identified the diarrheal disease agents most responsible for moderate-to-severe diarrheal disease and diarrhea-related mortality. The agents – enterotoxigenic and enteropathogenic *E. coli*, *Shigella* spp., rotavirus, norovirus, and *Cryptosporidium* spp. – are characterized by high infectivity, high fecal shedding, and transmission through a wide range of environmental reservoirs. This *Perspective* provides insight into the ecology of the diarrheal disease agents with emphasis on their relationship to environmental reservoirs. Based on this insight, the *Perspective* advocates for comprehensive interventions targeting exposure reductions across multiple environmental reservoirs. Single interventions are often inadequate. This may partially explain their failure to reduce environmental exposures below thresholds needed to initiate infection.

Introduction

Every year, there are an estimated 1.7 billion cases of gastrointestinal disease in children under five years old globally.[1] Of these, between 5 and 700 000 result in the death of the child.[1,2] These deaths are disproportionately experienced by people living in Low and Middle Income Countries (LMICs).[1,2] The reason is attributed to a combination of poverty, malnutrition, and living in remote areas with limited access to sufficient safe water, adequate sanitation, and health care.[3] Development efforts to reduce these risk factors globally have been largely successful: child deaths due to diarrhea have fallen dramatically – by 70–80% – from an estimated 2.5 million in 2000.[4] Despite dramatic reductions in diarrheal disease-related deaths (mortality), reductions in diarrheal episodes (morbidity) have declined only moderately. Between 1990 and 2010, diarrheal disease episodes declined only about 15%: from 3.4 to 2.9 episodes per child year.[5] The discrepancy between reductions in morbidity and mortality is due to emphasis on – and effectiveness of – therapeutic treatments such as oral rehydration therapy, zinc, and nutrient supplementation.[6] Therapeutic treatments improve recovery from infections but only reduce infection rates indirectly: by reducing pathogen shedding rates and durations. Vaccinations (*i.e.*, rotavirus) are also increasingly important

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interventions to reduce morbidity and mortality, though they target only a single cause of diarrhea.[6]

To improve both morbidity and mortality for all causes of diarrhea, environmental interventions – such as investments to improve water quality and quantity, sanitation, and food and hand hygiene – should be promoted. Public health improvements to interrupt environmental transmission of diarrheal diseases are largely credited as the cause of reductions of diarrheal diseases in developed countries.[7,8]

Interventions need to consider the importance of transmission through multiple environmental reservoirs.[11–13] Although single interventions may reduce exposures, the reduction may be insufficient to impact diarrheal disease prevalence. This is because there are multiple enteric pathogens transmitted *via* multiple exposure routes. Therefore, the impact of an intervention is dependent on the relative importance of the targeted transmission route.

The relative importance of transmission routes is likely site-specific.[13] And the factors influencing that importance are complex, including – at a minimum – etiology of endemic disease; seasonality; and water, sanitation, and hygiene infrastructure and practices.[14] This may help explain the observed variation in impacts of water, sanitation, and hygiene interventions.[11,15–20] This may also help explain observed multiplicative diarrheal disease reductions due to combined water and sanitation infrastructure.[11,19]

The theme of this *Perspective* is that greater reductions in diarrheal disease in LMICs can be achieved when interventions are designed based on knowledge about site-specific conditions – including etiology of disease and existing water, sanitation, and hygiene infrastructure and practices. Interventions should be designed to reduce aggregate exposures by targeting multiple environmental transmission pathways simultaneously. Efforts to identify one single, most effective, intervention – or to quantify a global mean for a single interventions' impact – are flawed in that the effectiveness of interventions varies by site. The theme is discussed through the lens of the characteristics of the most important diarrheal diseases (shedding, infectivity, growth, and persistence) and the general characteristics of environmental reservoirs (exposures, pathogen detection, and fecal contamination). Based on these characteristics, recommendations are made for designing packages of interventions to reduce diarrheal disease burden.

Diarrheal diseases in LMICs

Most diarrheal disease-related deaths are caused by only a handful of pathogens. Although the causes of diarrheal disease are wide and varied – including food allergies or intolerances, chemical or toxin exposures, and microbial infections – the vast majority of cases are caused by pathogens.[9,21] A systematic literature review of deaths due to diarrheal disease by Lanata *et al.* (2013) estimated that 70% (adjusted for age and to sum to 100%) are attributable to 13 pathogens.[21] The review highlighted the importance of five: rotavirus (17.8% of all deaths), enteropathogenic *E. coli* (14.0%), enterotoxigenic *E. coli* (7.3%), calicivirus (8.2%), and *Shigella* (6.4%).[21]

The results largely coincide with the Global Enteric Multicenter Study (GEMS), also published in 2013.[9] GEMS investigated etiological causes of moderate-to-severe diarrhea in children under five at seven sites across Africa and Asia. The authors were able to detect at least one of the 14 pathogens they tested for in 83% of all diarrheal cases; the cause of the other 17% of cases was not identified. GEMS also highlighted the importance of five – *Cryptosporidium* spp., *E. coli* producing heat stable toxin (an enterotoxigenic *E. coli*), typical enteropathogenic *E. coli*, rotavirus, and *Shigella* – as targets of interventions to "substantially reduce the burden of moderate-to-severe diarrhea".[9] Combining evidence from the work of Lanata *et al.* (2013) and Kotloff *et al.* (2013) suggests diarrheal disease prevention and treatment efforts should be focused on enterotoxigenic and enteropathogenic *E. coli*, *Shigella* spp., rotavirus, norovirus (an important calicivirus), and *Cryptosporidium* spp. (Table 1).[9,21]

Exposure, dose, and infection

For a child to be infected with a diarrheal disease, he must first be exposed. Exposure is describable as a continuous variable,

Table 1 Characteristics relevant to environmental transmission of important diarrheal diseases in low and middle income countries. PCR and RT-PCR are polymerase chain reaction and reverse transcription polymerase chain reaction, respectively. * – *Cryptosporidium* spp. shedding is in units of oocysts per day

Pathogen	Class	Shedding (#/g feces)	Duration (days)	50% infectious dose (#)	Human feces equivalents for infection (g)	Common detection methods	Hosts
Enterotoxigenic *E. coli*	Bacteria	10^7 to 10^8	3–5	10^5 to 10^8 cells	10^{-3} to 10^1	PCR	Humans, livestock, dogs
Enteropathogenic *E. coli*	Bacteria	10^5, 10^9 (peak)	>10	10^5 to 10^7 cells	10^0 to 10^2, 10^{-4} to 10^{-2} (peak)	PCR	Humans, livestock, dogs, cats
Shigella spp.	Bacteria	10^4 to 10^5, 10^6 to 10^{10} (peak)	7–14	10^3 cells	10^{-2} to 10^0, 10^{-7} to 10^{-3} (peak)	Isolation & biochemical profiling	Humans, closely related primates
Cryptosporidium spp.	Protozoa	10^3 to 10^7*	8 (2–35)	9–160 oocysts	10^{-6} to 10^{-1}	Microscopy & immunoassay	Mammals
Norovirus	Virus	10^7 to 10^8, 10^{12} (peak)	28	1320 genome equivalents	10^{-5} to 10^{-4}, 10^{-9} (peak)	RT-PCR	Humans
Rotavirus	Virus	10^5 to 10^{10}	24	6 focal forming units	10^{-9} to 10^{-4}	Immunoassay	Mammals, birds

meaning that a child may be exposed to a range of pathogens: from a few to many. Infection, however, is binary: a child is either infected or not. The likelihood that a child will be infected increases with increasing exposure, a pathogen-specific relationship describable by dose–response functions (Fig. 1). Dose–response functions relate dose to probability of infection (seroconversion and illness are also common endpoints). The pathogen dose at which there is a 50% likelihood of infection is known as the human infectious dose 50, or HID50. It is important to note dose–response functions are typically determined using adults, as opposed to children, and so may not accurately reflect infection risks for children.

To substantially reduce infections, tenfold reductions in exposures are often needed. The dose–response relationship links probability of infection – in arithmetic scale – to dose – in log-scale. So reductions of probability of infection from 50% to, for example, 10%, require a reduction in exposures of anywhere from 0.7 (*Cryptosporidium parvum*) to 1.5 (rotavirus) orders of magnitude (Fig. 1). Therefore, interventions that reduce exposures may not be sufficient to also reduce infections.

Of course, diarrhea is not the only consequence of environmental fecal exposures. Environmental bacterial exposures may also contribute to both malnutrition and stunting – which affected as many as 26% of children globally in 2011 – through enteric infections and/or environmental enteric dysfunction (EED).[10,22–24] Multiple ongoing research trials are examining links between environmental bacterial exposures and stunting. The Interactions of Malnutrition and Enteric Infections: Consequences for Child Health and Development (MAL-ED, NCT02441426) study is investigating the degree to which enteric infections (with and without diarrhea) contribute to undernutrition as mediated by intestinal inflammation and/or altered intestinal function.[10] The Sanitation, Hygiene, Infant Nutrition Efficacy Project in Zimbabwe (SHINE, NCT01824940) is investigating the impacts of water, sanitation, and hygiene (WASH) interventions alone and in combination with nutrition interventions on child health.[154] The hypothesis is that WASH interventions reduce fecal bacterial exposures, which reduces EED, thereby increasing intestinal functioning and improving nutrition interventions. Thirdly, the WASH Benefits trials in Bangladesh (NCT01590095) and Kenya (NCT01704105) are also assessing impacts of WASH and nutrition interventions on child health, including enteric infections, undernutrition, and EED.[25]

This *Perspective* focuses on enteric infections – as oppose to EED – because the causes and consequences of EED are currently uncertain. However, it is important to note that any reduction in microbial exposures may lead to an improvement in EED, in contrast to the binary outcomes of infection.

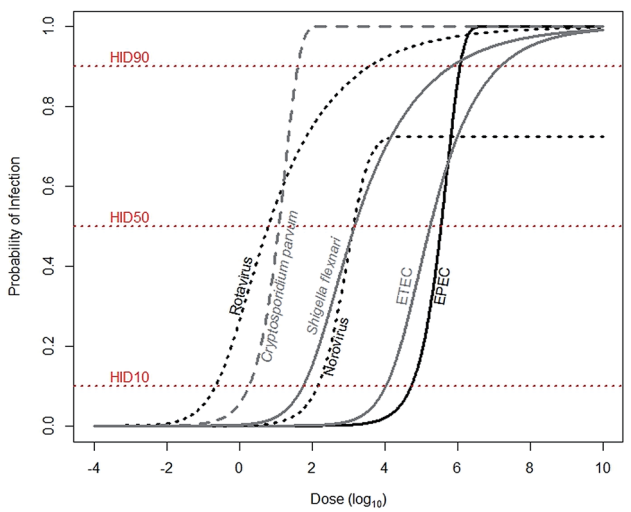

Fig. 1 Median estimates for dose–response relationships for common diarrheal diseases. Rotavirus ($\alpha = 0.253$, $N_{50} = 6.17$), *Shigella flexnari* ($\alpha = 0.265$, $N_{50} = 1.48 \times 10^3$), and ETEC ($\alpha = 0.375$, $N_{50} = 1.78 \times 10^5$) are described by beta-Poisson models, and *Cryptosporidium parvum* ($k = 5.72 \times 10^{-2}$) and EPEC ($k = 1.95 \times 10^{-6}$) are described by exponential models. Data are from either recommended (rotavirus, *S. flexinari*, and *C. parvum*) or most conservative (ETEC, EPEC) models described by the Quantitative Microbial Risk Assessment (QMRA) Wiki, curated and hosted by the Center for Advancing Microbial Risk Assessment (http://qmrawiki.canr.msu.edu). Norovirus ($\alpha = 2.910$ and $\beta = 2.734$) is described by an "aggregated exact beta-Poisson model" with assumed 27.54% immunity, based on Messner *et al.* (2015).[70] The dose at the intersection of the dose–response curves and the HID10, HID50, and HID90 lines corresponds to 10%, 50%, and 90% likelihood of infection, respectively.

Linking environmental contamination to probability of infection using human feces equivalents

Because substantial reductions in exposures are often needed to meaningfully impact infection, interventions should be designed to maximize reductions in pathogen exposures. Intervention impacts could be estimated using data on: (1) pathogen contamination of reservoirs, (2) type, intensity, and frequency of people–reservoir interactions, and (3) impacts of interventions on both (1) and (2). Unfortunately, these data are sparse, especially in LMICs, though ongoing efforts are seeking to remedy this issue (most notably, SaniPath Rapid Assessment Tool by the Center for Global Safe Water at Emory University, http://www.sanipath.org).[26,27]

In the absence of quantitative pathogen and human–environment interaction data, fecal contamination of environmental reservoirs can be linked to probability of infection using human feces equivalents. Human feces equivalents are a proxy measure to estimate exposure risks in the absence of quantitative pathogen data.[26,27] Here, this concept is applied to relate risks for multiple diarrheal disease agents to data on environmental fecal contamination. Human feces equivalents are estimated for both infection from diarrheal disease estimates and for environmental fecal contamination. Estimates for infection are estimated by dividing the HID50 – an indicator of a pathogen's infectivity – by the shedding rate – an indicator of pathogen density in feces:

$$\text{Human feces equivalents for infection(g feces)} = \frac{\text{HID50 (\# pathogens)}}{\text{shedding rate}\left(\frac{\text{\# pathogens}}{\text{g feces}}\right)}$$

Human feces equivalents for environmental fecal contamination are estimated by dividing *E. coli* concentrations within reservoirs by the average reported *E. coli* concentration in feces:

$$\text{Human feces equivalents for contamination (g feces)} = \frac{\text{contamination } (\#\ E.\ coli)}{E.coli \text{ in feces } \left(\frac{\#\ E.\ coli}{\text{g feces}}\right)}$$

E. coli is typically present in human feces at concentrations of around 10^5 to 10^{10} CFU per g feces; the lower end of the range (10^5 CFU g^{-1}) is used in this *Perspective* as a conservative choice.[28,29] It is important to note that human feces equivalents are intended as approximations. Values for HID50, shedding rates, *E. coli* contamination, and *E. coli* in feces are both uncertain and variable, so human feces equivalents will also be uncertain and variable.

Diarrheal disease agents

Enterotoxigenic (ETEC) and enteropathogenic (EPEC) *E. coli*. *E. coli* are highly-diverse, commensal organisms commonly found in the intestines of warm-blooded animals. High diversity allows *E. coli* to adapt to a range of environments. A recent study of *E. coli* genomes reflect this diversity: Lukjancenko *et al.* (2010) estimated that 90% of the *E. coli* pan genome and 80% of a typical *E. coli* genome are accessory – or non-essential – genes.[30]

ETEC are a pathotype of *E. coli* indicated by their mechanism of pathogenicity: colonization targeting intestinal mucosa combined with production of one or more enterotoxins.[31] Virulence determinants, which are primarily located on plasmids, are spread *via* horizontal gene transfer. So ETEC does not describe a "single homogenous group" but rather a cluster of strains from "multiple distinct lineages".[32,33] The high diversity amongst ETEC suggests that estimates of its transmissibility, persistence and growth, and infectivity based on experimental studies of a subset of strains may not be representative of the pathotype as a whole.

Nonetheless, from a handful of strain-specific studies, some consistency in ETEC characteristics is observed. Diarrheal disease caused by ETEC typically lasts 3–5 days though longer bouts have been documented.[34] In challenge studies, ETEC is shed in feces at concentrations as high as 10^7 to 10^8 CFU per g feces.[35–37] Infectivity of ETEC is strain-specific, but for all strains is relatively low: HID50 ranges from around 10^5 to 10^8 cells (http://qmrawiki.canr.msu.edu). Notable here is that the HID50 is equivalent to the estimated number of ETEC in 0.001–10 g feces of an infected person. ETEC can also cause infections in livestock (cattle, goats, pigs, and sheep) and other animals (dogs).

EPEC are a pathotype of *E. coli* characterized by their ability to produce attaching and effacing (A/E) lesions. Diagnosing EPEC relies primarily on molecular methods.[38] EPEC infections are indicated by the presence of *eae* – a gene which encodes the outer membrane protein intimin that mediates intestinal cell attachment – coincident with the absence of *stx1* or *stx2* – genes which encode Shiga toxin and indicate enterohemorrhagic *E. coli* (EHEC) infections.[38] From there, EPEC infections are classified as either typical or atypical based on the presence (typical) or absence (atypical) of the bundle-forming pilus structural gene *bfpA* located on the *E. coli* adherence factor plasmid (EAF).[38,39] Occasionally, the presence of the *bfpA* gene independent of *eaeA* is sufficient to diagnose typical EPEC, as in the GEMS study.[9,40] As EPEC infections can be indicated by the presence of a single gene located on a plasmid, it is not surprising that EPEC isolates also exhibit diversity across lineages.[41] EPEC infects other animals besides humans, including cattle, dogs and cats.

EPEC is characterized by substantially longer duration diarrhea than ETEC. EPEC diarrhea frequently lasts >10 days and is often associated with prolonged or persistent diarrhea.[42–44] EPEC is shed in feces at concentrations of around 10^5 cell equivalents per g feces, but can reach as high as 10^9 cell equivalents per g feces.[42] EPEC infectivity, like ETEC, is also strain-specific and relatively low: the HID50 is estimated to, be between 10^5 to 10^7 cells (http://qmrawiki.canr.msu.edu). This corresponds to the estimated number of EPEC in 0.01–1 g feces of an infected person.

***Shigella* spp.**. *Shigella* spp. are bacteria that are phylogenetically similar to *E. coli* – most of the *Shigella* spp. genes are shared with those of *E. coli* K12 strain MG1655, for example.[45] Phylogenetic relationships suggest that *Shigella* spp. are not necessarily a unique species, but rather a subspecies of *E. coli* that share a single pathovar with enteroinvasive *E. coli* (EIEC).[45,46] *Shigella* spp. are known to only infect humans and other closely-related primates. *Shigella* spp. are diagnosed through isolation and biochemical profiling.

Shigella serogroups *S. flexinari* and *S. sonnei* are responsible for the majority of infections. Within *Shigella*, there are four serogroups: *S. dysentariae*, *S. flexinari*, *S. boydii*, *S. sonnei*. In the GEMS study, the majority of *Shigella* infections (65.9%) were *S. flexinari*, followed by *S. sonnei* (23.7%).[47] Both *S. boydii*, and *S. dysentarie* were responsible for around only 5% of *Shigella* spp. infections.[47] However, *S. dysentarie* should not be overlooked as it is a causative agent of dysentary outbreaks responsible for both high attack rates and case fatality across all ages.[48]

Despite similarities to *E. coli*, *Shigella* spp. is substantially more infective. For almost all *Shigella* infections detected in GEMS, children were symptomatic with moderate-to-severe diarrhea.[9] The HID50 is estimated to be around 10^3 cells, though Levine *et al.* (1973) demonstrated that as few as 10 cells can cause illness (http://qmrawiki.canr.msu.edu).[49] *Shigella* infections typically last at least 7, and occasionally longer than 14, days.[50] *Shigella* counts per gram of feces typically range from 10^4 to 10^5, but reach concentrations of 10^6 to 10^{10} per gram at the height of excretion.[49,51,52] So the HID50 corresponds to the typical number of *Shigella* in 0.01–0.1 g of feces of an infected person, though may reach as low as 10^{-7} g feces at the height of excretion.

***Cryptosporidium* spp.**. *Cryptosporidium* spp. are protozoal pathogens with a complex lifestyle that includes the formation of oocysts that are released in feces into the environment. The oocysts are 4–6 μm in size and generally resistant to water treatment processes including disinfectants.[53,54] Both

microscopy and commercially available immunoassays are used for diagnosis.[40,55] GEMS identified *Cryptosporidium* spp. as the second most common pathogen in infants.[9] The species of *Cryptosporidium* spp. most relevant to humans are *C. hominis* and *C. parvum*, the latter of which can infect a broad range of mammalian hosts.

Voluntarily infected people shed oocysts at average concentrations of 10^3 to 10^7 oocysts per day.[56] Shedding continues for 2 to more than 35 days, with a median time of about 8 days.[57] *Cryptosporidium parvum* is highly infective, with an HID50 estimated to be as low as 9 oocysts (http://qmrawiki.canr.msu.edu).[58] Infectivity may be strain-specific, though, as other strains have HID50s of estimated at around 160 oocysts (http://qmrawiki.canr.msu.edu).[59] So the HID50 for *Cryptosporidium* spp. corresponds to 10^{-1} to 10^{-5} of the amount of feces shed in a day during an infection.

Rotavirus. Rotavirus is arguably the most important enteric pathogen globally, despite its small size. Nearly everyone is infected with rotavirus at an early age.[60] The virus is only about 80 nm in diameter and encapsulates 11 segments of double stranded RNA. Six of the segments encode structural proteins, one of which (VP6) forms the majority of the capsid and is used as the target of immunoassays for diagnostics.[61] Rotavirus is highly diverse, with eight known species (A–H). One (A) accounts for more than 90% of human infections, though B and C are also known to infect humans. Rotavirus is also a zoonosis, as species A, B, and C can also a broad range of mammals and birds.

High shedding rates combined with high infectivity contribute to rotavirus's ubiquity. Approximately 10^3 to 10^{10} genome copies of rotavirus per g feces is shed during infection.[62,63] In a study of ten symptomatic children in Vellore, India, viral shedding lasted a median of 24 days.[64] Rotavirus infectivity is very high: the HID50 was identified as 6 focus-forming units (FFU) in the most well-known study by Ward *et al.* (1986).[65] Relating FFU to genome copies using a conservative ten-fold estimate, the HID50 is equivalent to a range of 10^{-3} to 10^{-9} g feces of an infected person.

Norovirus. Although rotavirus is endemic, norovirus is characterized more by its role in sporadic outbreaks. Approximately 90% of all epidemic outbreaks of nonbacterial gastroenteritis are attributable to norovirus.[66] The virus is 20–40 nm in diameter and encapsulates a positive-stranded RNA genome. Norovirus, and in particular norovirus genogroup GII, is characterized by a high mutation rate due to reduced polymerase fidelity.[66] The high mutation rate likely contributes to the rapid emergence and spread of norovirus variants over the last 20 years.[66] Norovirus infections are typically diagnosed using reverse transcriptase PCR targeting capsid proteins specific to genogroup GI or GII. Although there are animal strains of norovirus, zoonotic potential is currently thought to be low.[67]

Norovirus is characterized by high infectivity, but some people are naturally resistant. Norovirus infectivity is unique in that it infects only people with histo-blood group O or A with a functional FUT2 enzyme ("secretor-positive").[68] Based on this finding, a volunteer challenge study estimated that norovirus HID50 is approximately 1320 genome equivalents for susceptible people.[69] Subsequent analysis of challenge studies suggested that immunity rates (non-susceptible people) make up about 27% of the study population.[70] These values remain debatable, however: Schmidt *et al.* (2015) contend the HID50 may be overestimated due to limited information on both aggregation of virus and immunity status of volunteers due to other factors, like prior exposures.[71]

Nevertheless, in those infected with Norwalk virus, the prototype norovirus strain, norovirus shedding in feces ranged from 10^7 to 10^{12} genome copies per gram and lasted a median of 28 days.[72] Viral loads for natural infections are reportedly within the lower end of the range (10^7 to 10^8).[73,74] Assuming the HID50 estimate of 1320 genomes, the HID50 is equivalent to approximately 10^{-4} to 10^{-5} feces during a natural infection.

Environmental transmission

We can conceptualize the environmental transmission of diarrheal diseases using the F-diagram (Fig. 2). The F-diagram visualizes the role of five or six environmental reservoirs (typically fields, fingers, fluids, flies, food, and sometimes fomites) in diarrheal disease transmission from infected to susceptible people. Pathogens are spread from feces into one or more of the environmental reservoirs through human–environment interactions, animal–environment interactions, and/or natural processes. Subsequent interactions with the reservoirs by susceptible people can result in infection.

Fluids. Drinking water is arguably the most efficient exposure pathway. The average child and adult consume a median of 0.2–0.5 and 0.8–1.2 liters of water each day, respectively.[75] And fecal contamination is common: Onda *et al.* estimated that 23% of the global population used unsafe water in 2012 based on

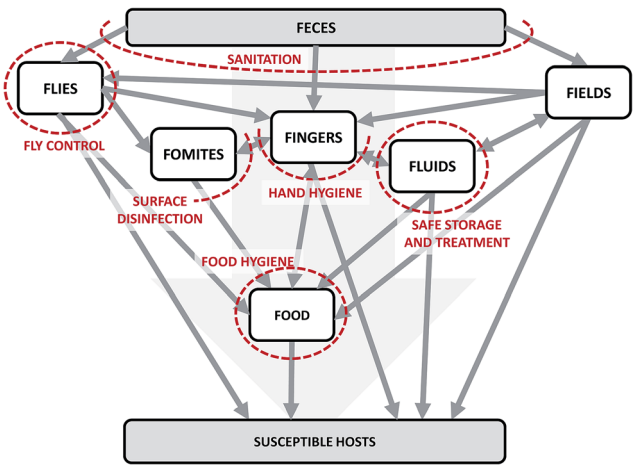

Fig. 2 The F-diagram, a conceptual model of the complexity of potential transmission pathways of diarrheal diseases through environmental reservoirs. Adapted from Wagner and Lanoix (1958) and Kawata (1978).[12,13] Grey arrows represent potential pathways of pathogen transmission. Red dashed lines represent the impacts of interventions on intersecting arrows. The lines are dashed to imply permeability due to incomplete effectiveness (fly control, food hygiene, hand hygiene, sanitation) and/or imperfect compliance (food hygiene, hand hygiene, safe storage and treatment, and sanitation).

presence of thermotolerant coliforms and results of sanitary surveys.[76]

Even if source water is safe, stored water may not be. Contaminated storage containers, hands, drinking cups, and other utensils that come into contact with stored water are responsible for degradation of water quality within a home.[77,78] As water is a shared resource, water contaminated by one member of the household puts others at risk. The diarrheal disease agents EPEC, ETEC, *Shigella*, rotavirus, norovirus, and *Cryptosporidium* spp. have all been detected in stored drinking water in LMICs.[78–85]

Drinking water is likely an efficient route of transmission for rotavirus, norovirus, and *Cryptosporidium* spp.; less so for ETEC, EPEC, and *Shigella* spp. due to the relatively high HID50s for the bacterial pathogens. In a study of Tanzanian children, Mattioli *et al.* (2015) estimated median daily fecal consumption through drinking water of 10^{-4} to 10^{-6} g feces.[26] This range overlaps estimates for feces equivalents of the HID50s for norovirus, rotavirus, *Cryptosporidium* spp. and *Shigella* spp. during peak shedding, but is orders of magnitude lower than the HID50 feces equivalent for ETEC, EPEC, and *Shigella* spp. given typical shedding rates.

Of note, the WHO guidelines for drinking water do not necessarily indicate an absence of pathogens. The current guideline states that *E. coli* or thermotolerant coliform must not be detectable in 100 ml samples.[86] This standard (<1 CFU *E. coli* per 100 ml) is equivalent to approximately 10^{-5} to 10^{-10} g human feces. Water in households where there is shedding of rotavirus, norovirus, *Cryptosporidium* spp., or *Shigella* spp. could be sufficiently contaminated to cause disease even when there are no *E. coli* detectable.

Although contamination of water needs to be fairly high to risk exposure to the HID50 for the bacterial pathogens EPEC, ETEC, and *Shigella*, bacteria can increase during storage through growth. *E. coli*, including enterotoxigenic *E. coli*, can grow in drinking water distribution systems and persist in freshwater for up to 3 months.[87–89] In stored drinking water containers, risk factors for growth of total coliform – of which *E. coli* is a member – include hand contacts, presence of biofilms, high temperature, and high assimilable organic carbon (AOC).[79,90] So bacterial pathogen growth is primarily a concern when water with high AOC is stored at warm temperatures for long periods of time.[90]

Food. Similar to drinking water, food is also an efficient transmission pathway. Fecal contamination can occur pre-harvest, during preparation for sale, or within the home.[91–95] Pre-harvest contamination can occur due to use of biosolids, animal or human manure, or untreated irrigation waters during farming.[96] Fecal contamination levels on food are highly variable, reflective of the variability in processing. In Tanzania and Ghana, *E. coli* on the surface of produce varied from undetectable to more than 10^5 CFU per piece.[92,94] In Bangladesh, more than 10^2 CFU fecal coliform per g complementary feeding foods were detected when the food was first prepared.[93] These concentrations correspond to approximately 10^{-3} to 10^0 g human feces equivalents. EPEC, ETEC, and *Shigella* spp. have all been detected on foodstuffs.[93,96,97]

The greatest risk factor for foodborne transmission of pathogens is the ability of bacterial pathogens to grow. Both *Shigella* spp. and *E. coli* are capable of growing rapidly in various food stuffs, including cheese, rice, milk, and beef.[98] *Shigella flexinari* and EPEC for example, reached concentrations between 10^5 to 10^8 CFU per g or CFU per ml in these matrices when incubated at 25 °C.[98,99] Lower temperature storage *via* refrigeration reduces growth, but refrigerator ownership is generally low in LMICs.[100] Accounting for growth, food is an efficient carrier of the equivalent of 1–100 g feces for bacterial pathogens.

Fingers. High concentrations of fecal bacteria are frequently detected on hands in LMICs. Geometric means reported for *E. coli* contamination on hands range from 1–3.5 $\log_{10}$ CFU/2 hands *E. coli* and enterococci, for example.[101,102] Studies that have looked for diarrheagenic pathogens have noted the presence of multiple *E. coli* pathotypes including ETEC and EPEC, *Shigella* spp., rotavirus, and norovirus.[83,101,103–106] Once on hands, pathogens can survive for long periods of time. Rotavirus, for example, survives for more than 260 minutes.[107]

Hand contamination poses both direct and indirect diarrheal disease risks. Here, direct risks refer to direct exposure from hand-to-mouth contacts. Children and adults touch their mouths approximately 3–28 and 8 times per hour, respectively.[75] The frequency of contacts drives risks because anywhere from 33–41% of microbial contamination can be transferred from hands-to-mouth on a single contact.[108] Based on *E. coli* and enterococci contamination on hands in Tanzania, Mattioli *et al.* (2015) estimate that children consume the equivalent of a median 10^{-3} to 10^{-4} g feces per day due to hand–mouth contacts.[26] Hands are also responsible for indirect risks. Drinking water, food, and fomites are often contaminated due to contacts with contaminated hands.

Hands are likely an efficient route of rotavirus, norovirus, *Shigella* spp., and *Cryptosporidium* spp. transmission due to the high infectivity of these diarrheal pathogens. This is also observed in developed countries, where epidemiological surveys have suspected outbreaks were due to poor hand hygiene, often during food preparation.[109–112] Direct transmission of ETEC and EPEC by hands is likely only when hands are heavily contaminated. During outbreaks, for example. The exposure estimates by Mattioli *et al.* (2015) border the estimated HID50 feces equivalents for these pathogens.[26] However, the role of hands in indirect bacterial pathogen transmission is likely very important. Transfer of EPEC, ETEC, and *Shigella* spp. to environmental reservoirs where they can grow (like food), may contribute substantially to diarrheal disease burden.

Flies. Flies are important reservoirs for enteric pathogen transmission due to their attraction to both feces and food. Flies attracted to feces pick up pathogens through direct contact (mechanical transport) or consumption of the feces (either as flies or earlier, as larvae).[113] The best evidence of this is the frequent detection of enteric pathogens on flies, including *Cryptosporidium*, ETEC, *Shigella*, norovirus, and rotavirus.[114,115] The flies then move the feces into the environment through some combination of mechanical transport, regurgitation, and/or defecation.[116]

Quantitative data on pathogen contamination of flies is scarce, making it difficult to determine the role of flies in disease transmission. On a United States cattle farm in Kansas – admittedly very different than households in LMICs – Alam *et al.* (2004) estimated that approximately 1–3% of flies carried 10^1 to 10^5 CFU *E. coli* 0157:H7, an enterohemorrhagic *E. coli* strain.[117] De Jesús *et al.* (2004) estimated that flies are capable of contaminating surfaces with bacteria from the equivalent of about 10^{-4} g of food.[118] Extrapolating these results to feces suggests that flies may be effective carriers of infectious doses of rotavirus, norovirus, *Cryptosporidium* spp., and *Shigella* spp. but that the doses may be lower than would be needed for effective transmission of EPEC or ETEC.

But even though flies may not be carriers of infectious doses of EPEC or ETEC, flies provide opportunities for these bacteria to grow. Studies demonstrated that *E. coli* could grow on the surface of flies and in regurgitation spots, though the latter phenomenon was only observed for *E. coli* artificially deposited on regurgitation spots.[116,119] Similarly, *E. coli* and *Shigella* transferred to food are capable of growing, as discussed earlier.

Fields. In the context of the F-diagram, fields refers to crops and soils.[13] Fields are contaminated through land application of human excreta and/or animal manure (potentially for growing crops) and open defecation of humans and/or animals. In soils in a community around Accra, Ghana, *E. coli* concentrations ranged from 10^{-2} to 10^5 CFU per g soil.[120] Norovirus was also detected in a subset of samples. The primary role of fields in transmission of diarrheal diseases in this context is as an intermediate reservoir. Manure on agricultural fields contaminates food (*i.e.*, crops); stormwater run-off contributes fecal contamination from open defecation to surface waters.

Recent evidence, however, has suggested that fields should be expanded to include flooring inside and near households. *E. coli* concentrations ranged from a mean of 10 to 10^3 CFU per g soil in household plots in Zimbabwe and Tanzania.[92,121] In Tanzania, *E. coli* pathotypes including EPEC and ETEC were also detected. Though the fecal source is uncertain, suggestions include inadequate management of animal feces, child feces, and/or wastewater, as well as off-plot fecal bacteria sources.[92] Growth is also a risk for bacterial pathogens, as *E. coli* have been shown to grow in soils.[122]

Fecal contamination of soil in the household is a transmission concern for all diarrheal pathogens. In Zimbabwe, Ngure *et al.* (2012) estimated that infants consume the equivalent of 1 g of chicken feces (coprapaghy) and 20 g of soil (geophagy) daily.[121] Copraphagy alone would ensure infection with any zoonotic diarrheal diseases – including ETEC, EPEC, and rotavirus – if the animal is shedding. Under the assumption of proper animal fecal management, children would still be exposed to the equivalent of 10^{-3} to 10^{-1} g of feces per day through geophagy.

It is important to note, however, that the role of soil around households in disease transmission is also site-specific. For example, Ngure *et al.* (2012) estimates of soil ingestion are 500 times greater than estimates for children in the United States.[75] Similarly, there was a ten-fold difference in *E. coli* contamination of household soils between Tanzania and Zimbabwe; there is likely greater variation at other sites. Nevertheless, soil contamination is a transmission concern given infant and young child interactions with flooring.

Fomites. A fomite is any inanimate object capable of transmitting diseases. In LMICs, there is extensive evidence of fecal contamination (namely *E. coli*, and thermotolerant coliform) on fomites, including dishes in Peru, dish cloths in South Africa, children's toys in Bangladesh, Honduras, and rural India, and throughout households in Cambodia and Tanzania.[92,123–129] In studies of *E. coli*, contamination levels range dramatically, between 1 and 100 CFU/100 cm^2, and occasionally exceeding 1000 CFU/100 cm^2 – especially on wet surfaces.[92,127] This level of contamination corresponds to 10^{-3} to 10^{-5} g feces and occasionally more than 10^{-2} g feces. In addition to fecal bacteria, pathogenic *E. coli* including EPEC and ETEC, were detected in the studies of dish clothes in South Africa and surfaces in Tanzania.[92,127]

Once contaminated, fomites readily transfer pathogens to other surfaces. Transfer events move a fraction of the pathogen (typically from <0.01–50%) between the fomite and other reservoirs.[130] The magnitude of the fraction is dependent on pathogen, fomite, hand or other surface characteristics, and environmental (*i.e.*, temperature, humidity) characteristics.[130,131]

Compounding risks of fomite transmission is the ability of pathogens to persist on surfaces for extended periods of time. Persistence of *Shigella* spp., and *E. coli* ranges from 1.5 days up to 16 months.[132] Norovirus and rotavirus persist for at least 2 months.[133] In contrast, *Cryptosporidium parvum* persists on surfaces for less than two hours.[134] Factors that influence persistence are similar to those that influence transfer: material type, humidity, and temperature.[132–134]

Given the modest fecal contamination levels observed on fomites, transmission concerns are primarily for rotavirus and norovirus, and to a lesser extent *Shigella* spp. following peak shedding. Given *Cryptosporidium* spp. susceptibility to desiccation, it is unsurprising that evidence of *Cryptosporidium* spp. transmission by fomites is sparse. Fomite-mediated transmission for bacterial pathogens is likely relevant primarily as an intermediate prior to transfer to other reservoirs where subsequent growth is a concern (like food).

Discussion

This *Perspective* argues that greater reductions in diarrheal disease in LMICs can be achieved when interventions are designed based on site-specific conditions to interrupt multiple transmission routes. Systems-based approaches to interventions are needed to further reduce diarrheal disease burden in LMICs.[11] The diarrheal diseases most important for child health – enterotoxigenic and enteropathogenic *E. coli*, *Shigella* spp., rotavirus, norovirus, and *Cryptosporidium* spp. – are characterized by high infectivity, high fecal shedding, and transmission through a wide range of environmental reservoirs (Table 1, Fig. 2). There is likely no single intervention that will universally and uniformly reduce diarrheal diseases globally. Interrupting a single transmission route may reduce total exposure, but other pathways may still contribute sufficient exposure to cause

infection. Packages of interventions should be designed to interrupt simultaneously all of the relevant transmission pathways to sufficiently reduce infections. The most effective intervention packages are site-specific. Characteristics that influence intervention effectiveness include diarrheal disease etiology and existing water, sanitation, and hygiene infrastructure and practices.

Investment in combined interventions does not necessarily lead to interruption of multiple exposure pathways. Previous reviews have failed to demonstrate additive impacts of combined interventions on diarrheal disease.[15,135,136] There are several hypotheses as to why combined interventions have failed to show health improvements. One review – by Fewtrell *et al.* (2005) – suggested the lack of additive impacts of combined interventions on health may be due to incomplete or inconsistent implementation.[15] Related to incomplete implementation, Enger *et al.* (2013) showed that imperfect user compliance strongly influences intervention effectiveness.[137] This *Perspective* contributes an alternative explanation, that standard interventions may be redundant because they impact similar transmission pathways while others are neglected.

To avoid redundancies, the transmission pathways for the main pathogens responsible for diarrheal diseases should be considered. For example, this *Perspective* suggests that bacterial pathogens (EPEC, ETEC, and *Shigella* spp.) can be controlled through reducing geophagy (consumption of contaminated soil),[121] prevention of growth in food,[138] and – especially in the context of *Shigella* spp. – fly control.[139,140] Given reported high rates of geophagy for soil flooring, it should be unsurprising that the upgrade of concrete flooring alone reduced diarrheal disease incidence by 13% during the Piso Firme Program in Mexico.[121,141] Hand hygiene is also important for control of bacterial pathogens due to its reduction of both direct hand-to-hand contact transmission and – perhaps more importantly – physical transfer of bacteria to reservoirs where growth is possible.

Cryptosporidium spp. and norovirus are more difficult to control than bacterial pathogens due to the combination of low infectious doses and high shedding rates (Table 1, Fig. 3). Interventions targeting *Cryptosporidium* spp. or norovirus need to interrupt all exposure routes, through (for example) animal, child, and adult fecal management; dedicated safe water treatment and storage; hand hygiene; and limiting contacts with infected household members. Notably, child feces was described as "the most important contaminant in the household environment with the highest risk of exposure to young infants" in a 2004 review of infant and young child feces management.[142] When a person inside the home is infected with *Cryptosporidium* spp. or norovirus, others are also at an increased risk (between 2- to 26-times), so limiting household contacts is also important.[143–145]

Unfortunately, preventing rotavirus infection is nearly impossible. The high infectivity combined with a high rate of shedding require near complete avoidance of the infected person (Fig. 3). It is therefore unsurprising that nearly everyone is infected by the age of five, including children in industrialized countries.[60,146] Efforts to reduce rotavirus-related child morbidity and mortality should be focused on vaccination,

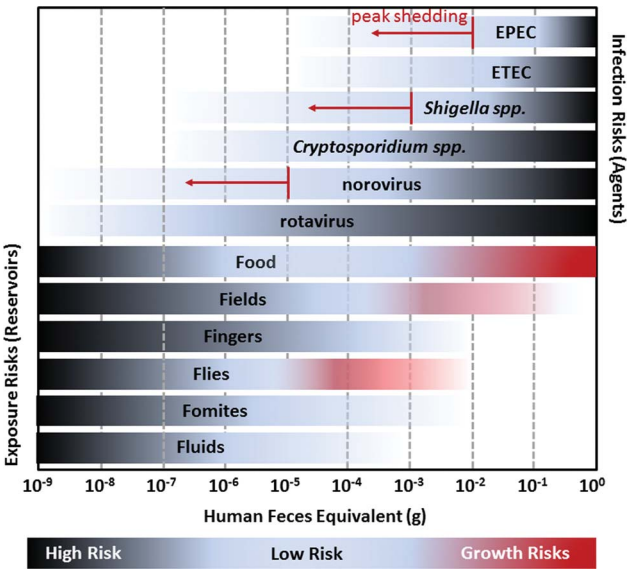

Fig. 3 A visualization of the relationship between the estimated amount of human feces equivalents required to initiate infection for diarrheal pathogens (EPEC, ETEC, *Shigella* spp., *Cryptosporidium* spp., norovirus, and rotavirus) and the approximate amount of fecal contamination observed in environmental reservoirs (Food, Fields, Fingers, Flies, Fomites, and Fluids). The human feces equivalents for infection risks are based on the ratio of the 50% human infectious dose (HID50) to the number of pathogens shed in feces. For example, the HID50 for *Shigella* spp. is approximately 1000 cells and *Shigella* spp. is typically shed in feces at concentrations of 10^4 to 10^6 cells per g, so infection risks are highest for people exposed to more than $\sim10^{-3}$ g feces of an infected person. However, *Shigella* spp. may reach as many as 10^{10} cells per g during peak shedding so there is a non-negligible risk of infection for exposure to more than 10^{-7} g feces. Darker shading for pathogens refers to increased risk of infection; when exposed to a higher feces equivalent there is a greater likelihood of infection. Risks from peak shedding are indicated by red arrows. Fecal contamination in environmental reservoirs is estimated from the ratio of *E. coli* contamination on surfaces as reported in previous studies to *E. coli* concentration of feces (conservatively estimated as 10^5 colony forming units per g). Darker shading for environmental reservoirs refers to increased likelihood of contamination: it is more likely that a reservoir will have 10^{-9} g feces than 10^{-8} g. For Food, Fields, and Flies, the possibility of, bacterial pathogen (EPEC, ETEC, and *Shigella* spp.) growth can further increase risks; this is depicted by red shading.[12,13]

nutrition, safe and plentiful water, and health care including oral rehydration therapy.[60] The goal should be to delay infection until after a child is 1 year old when likelihood of hospitalization and risk of mortality are reduced.[60]

Primary data on etiology of diarrheal disease is expensive and technically challenging in many settings. Expenses increase when accounting for geographic and seasonal changes in pathogens.[9,147–149] In the absence of primary data, secondary sources may provide guidance for intervention design. Examples of secondary sources include data obtained from local clinics and hospitals or from prior studies (*e.g.*, GEMS and MAL-ED) from similar (*e.g.*, socioeconomic status, climate, geography) sites. Development of low cost clinical diagnostic tools, epidemiological studies to identify risk factors for specific

causes of diarrhea, and infectious disease modeling[14,150] may provide additional data on etiology when other sources are not available. It is important to note that imperfect information on important causes of diarrhea is likely more useful to intervention design than no information. Researchers should also consider monitoring child diarrheal disease infections as indicators of child health as opposed to diarrheal disease generally.[151]

Interventions also need to consider prevailing water, sanitation, and hygiene infrastructure and practices. Previous research has shown intervention effectiveness varies by location and by water, sanitation, and hygiene conditions.[11,152] Information on water, sanitation, and hygiene is generally available at the country-level (*i.e.*, Demographic Health Surveys, Multiple Indicator Cluster Surveys), but more site-specific data can be obtained through deployment of additional surveys, such as the WHO's Core Questions on Drinking Water and Sanitation for Household Surveys.[153] Given the diversity of diarrheal disease agents and the mounting evidence regarding the importance of multiple environmental reservoirs in transmission, observed variation in intervention efficacy should not be surprising.[11,15–20] Greater reductions in diarrheal disease in LMICs can be achieved when the design of interventions is informed by site-specific conditions – including etiology of disease and existing water, sanitation, and hygiene infrastructure and practices.

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