Title

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Journal

Proceedings of the National Academy of Sciences of the United States of America, 115(12)

ISSN

0027-8424

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Publication Date

2018-03-20

DOI

10.1073/pnas.1719579115

Modeling environmentally-mediated rotavirus transmission: the role of temperature and hydrologic factors

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This manuscript was compiled on January 18, 2018

Rotavirus is considered a directly transmitted disease due to its high infectivity. Environmental pathways have, therefore, largely been ignored. Rotavirus, however, persists in water sources, and both its surface water concentrations and infection incidence vary with temperature. Here, we examine the potential for rotavirus to exploit water as a transmission pathway. We employ a mechanistic model that incorporates both direct and waterborne transmission pathways, coupled with a hydrological model, and we simulate rotavirus transmission between two communities with interconnected water sources. To parameterize temperature dependency, we estimated temperature-dependent decay rates in water through a meta-analysis. Our meta-analysis suggests that rotavirus decay rates are positively associated with temperature (N=39, p$<$0.001). This association is stronger at higher temperatures (over 20°C), consistent with tropical climate conditions. Our model analysis demonstrates that water could disseminate rotavirus between the two communities for all modeled temperatures. While direct transmission was important for disease amplification within communities, waterborne transmission could also amplify transmission. In standing water systems, the modeled increase in decay led to decreased disease, with every 1°C increase in temperature leading to up to a 2.4% decrease in incidence. These effect sizes are consistent with prior meta-analyses, suggesting that environmental transmission through water sources may partially explain the observed associations between temperature and rotavirus incidence. Waterborne rotavirus transmission is likely most important in cooler seasons and in communities that use slow-moving or stagnant water sources. Even when indirect transmission through water cannot sustain outbreaks, it can seed outbreaks that are maintained by high direct transmission rates.

Rotavirus | Environment | Temperature

Diarrheal disease is the fifth leading cause of mortality worldwide and the third leading cause of death among children under five. The World Health Organization estimated that diarrheal disease accounted for 2.9% of the Disability Adjusted Life Years (DALY) burden worldwide in 2015, making it the sixth leading contributor to the burden of disease (1). A recent multi-country study on diarrheal disease etiology showed that rotavirus was one of four pathogens that together accounted for the vast majority of severe diarrheal disease (2). The recent introduction of rotavirus vaccines has reduced the prevalence of severe cases but is less likely to impact the overall transmission rate, especially given variable coverage of the rotavirus vaccine in low- and middle-income countries, as well as lower vaccine efficacy in the developing world (3, 4). As such, rotavirus is likely to remain an important pathogen for the foreseeable future.

Like other enteric pathogens, rotavirus can be spread either directly from person to person or through contact with contaminated water. However, because of its low infectious dose and high shedding rate among infected individuals, environmental control is generally not considered effective for rotavirus (5) and indirect transmission through water is often not considered when analyzing rotavirus transmission dynamics (6–10). Instead, researchers have generally assumed that transmission through contaminated water is negligible in comparison with transmission occurring within homes and from person to person (with fomites as an intermediary).

In the tropics, meta-analyses have estimated that every 1°C increase in temperature is associated with a 4–10% decrease in rotavirus-associated diarrheal disease incidence (11, 12). Given that there is some evidence of publication bias for the temperature rotavirus effect and that other seasonal processes may affect risk, the true association may be closer to the lower end of this range (12). In temperate regions, researchers have seen that rotavirus is more common during the cooler weather months (13). A number of studies have examined fomite transmission, and although rotavirus can survive on fomites for long periods of time (on the order of weeks), there is a lack

Significance Statement

Although rotavirus persists in water and its incidence varies with temperature, the potential role of waterborne transmission to explain this seasonal pattern in low income countries has thus far been neglected. We find that water can affect the size of outbreaks for larger communities that draw water from slow-moving or stagnant sources and is likely most important in cooler seasons. Water can also spread outbreaks between communities, even when environmental transmission cannot sustain outbreaks. Our model may help explain temperature-incidence associations found in prior meta-analyses in the tropics, where humidity is higher year-round, and shows that the effect of temperature on risk depends on local hydrologic conditions. These findings provide insight into why the effect of temperature might be context-specific.

A.N.M.K. conceived of the study and analytic approach, carried out all analyses, and drafted the manuscript; N.L. performed the literature review, helped conduct the meta-analysis, and helped draft the manuscript; A.F.B. helped design the transmission model analysis and helped draft the manuscript; P.A.C. helped design the meta-analysis approach and helped draft the manuscript; J.V.R. helped design the meta-analysis approach and helped draft the manuscript; J.N.S.E. conceived of the study and helped draft the manuscript.

We have no conflicts of interest to report.

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of evidence that temperature has a strong effect on rotavirus persistence on fomites (14–18). In low- and middle-income countries in tropical settings, an alternative environmental pathway, such as water, may contribute to the temperature-driven incidence patterns. In temperate climates, seasonality in direct transmission is more likely to be explained by large seasonal shifts in relative humidity, with drier conditions promoting more direct transmission (14–18).

Various studies have attempted to predict the mechanisms by which increased temperature might alter risk of diarrheal disease by changing pathogen survival and growth dynamics, pathogen virulence, and human behavioral factors (19). In general, the burden of diarrheal disease is expected to increase as climate change progresses (20); however, there is a great deal of heterogeneity by pathogen (12). For bacterial pathogens, increased temperature is related to decreased persistence but can also lead to expression of genes associated with increased virulence (21, 22), and the net effect of increased temperature is thus expected to increase risk. In contrast, higher temperatures are only expected to decrease environmental persistence of viruses, including rotavirus (19), and thus are expected to reduce viral infection risks (11). Although this body of work has helped establish the associations between environmental factors and disease transmission risk, studies are needed to better elucidate the mechanisms by which environmental changes, such as increased temperature, affect risk (23).

In this study, we use a mechanistic model of rotavirus transmission to evaluate the potential of rotavirus to be transmitted through the environment. To parameterize this model and to assess how temperature might impact this pathway, we conduct a meta-analysis of experimental data to derive a statistical model for the relationship between temperature and rotavirus persistence in water. We then apply the resulting temperature sensitivity function within our rotavirus transmission model in two communities linked by a flowing body of water, examining the potential for waterborne transmission to disseminate rotavirus infections between communities and amplify cumulative incidence within communities. The hydrologic conditions in these two communities were parameterized to reflect the conditions found in our study site in rural, coastal Ecuador (24), which encompasses three rivers with distinct average flow velocities: the Onzole (17 km/day), the Cayapas (26 km/day), and the Santiago (69 km/day) (25). We also assess how changing temperature might impact risk at both the community and regional scales and explore how local hydrologic conditions and access to safe water and sanitation services could impact this relationship. The aim of this analysis is to estimate how temperature-related changes in waterborne transmission, mediated by the waterborne decay rate, might help explain correlations between rotavirus incidence and temperature or season, especially in tropical settings.

Results

The Basic Reproduction Number. Using the next-generation approach, we derived the following expression for $\mathcal{R}_0$ in one community (see supplement):

$$R_0 = \frac{\beta_H}{\gamma} + \frac{0.5\beta_W N\rho\dfrac{(1-c)\phi}{V}}{\gamma(\mu+\nu)} \quad [1]$$

$$= R_{0,H} + R_{0,W} \quad [2]$$

In equation 1, β_H is the transmission rate for direct, human-to-human transmission, γ is the recovery rate, β_W is the transmission rate from water, N is the population size, ϕ is the shedding rate for infected individuals, ρ is the water consumed per day, $1-c$ is the fraction of shed pathogens that ultimately mix in the local water supply, μ is the temperature-dependent rotavirus decay rate in water sources, and ν is the river flow velocity. See Table 2 for more details about the parameters. The basic reproduction number can be written as a sum of two submodel reproduction numbers (equation 2) $\mathcal{R}_{0,H}$ (the $\mathcal{R}_0$ from human-to-human transmission) and $\mathcal{R}_{0,W}$ (the $\mathcal{R}_0$ for water-to-human transmission).

This expression leads to several insights. First, when all transmission is person-to-person ($\beta_W = 0$), the reproduction number reduces to the familiar SIR form of β/γ, which is the general formulation assumed by other rotavirus transmission models. Second, for water-to-human transmission, the river flow velocity impacts how strong of an effect temperature has on $\mathcal{R}_{0,W}$. For flowing water systems ($\nu > 0$) temperature related die-off ($\mu = K(T)$) has a negligible effect whereas in standing water ($\nu = 0$), temperature related die off becomes more important for determining transmission potential. Third, the river flow velocity also impacts persistence in the local water supply. In flowing systems, the speed of flow is generally much faster than the speed of pathogen decay. As such, the length of pathogen persistence in the *local* water supply is much more strongly affected by hydrological flow dynamics. To facilitate comparison between these two rates for the dissemination analysis, we fixed the distance of each river compartment, scaling each compartment by its length (see supplement for details). Thus, flow velocity and decay rates are on the same scale in our model (units of day^{-1}). In standing water systems, the length of pathogen persistence in the environment is entirely driven by temperature. Even in standing water, however, the most important contributor to water-human transmission ($\mathcal{R}_{0,W}$) is the size of the water reservoir (V) relative to the effective shedding rate $(1-c)\phi$. Overall, the size of the water reservoir relative to the human population is the critical driver of the strength of water-to-human transmission, as it determines the degree of dilution that occurs when pathogens are shed into the water.

Meta-Analysis: Temperature and Die Off. Our literature search returned 57 articles. Of these articles, we identified 9 that met our inclusion criteria containing a total of 39 experiments for use in our analysis. These studies are summarized in Table 1. We used data from these 39 experiments to estimate the die-off rate (day^{-1}) for each study. The decay rates ranged from 0.008 to 0.996 per day, with a median decay rate of 0.056/day (corresponding to a median survival time of 18 days).

Our pooled data across all 39 experiments were fit by general linear regression,

$$\log(K(T)) = \beta_0 + \beta_1(T-25) + \beta_2\chi_{\text{nat}}, \quad [3]$$

and support the hypothesis that the decay rate $K(T)$ of rotavirus is temperature (T) dependent, with higher temperature

Table 1. Studies included in the meta-analysis. The phrase 'combined' is used for simple aqueous solutions at a neutral pH, such as phosphate-buffered saline (PBS).The phrase 'purified' is used for sterile, distilled water. The phrase 'treated' is used for water taken from raw water or sewage that was treated to remove microbes prior to analysis. More details about the estimated decay rates from each experiment are in the supplement.

Author, Year	No. Experiments	Temperature	Media Type	Ref
Espinosa et al., 2008	4	15, 25°C	Natural, Combined	(26)
Ward et al., 1986	9	4, 16, 23, 27, 29, 37°C	Natural	(27)
Raphael et al., 1985	12	4, 20°C	Natural, Treated	(28)
El-Sanousy et al., 2014	3	4, 22, 35°C	Natural	(29)
Hansen et al., 2007	1	22°C	Treated	(30)
Sattar et al., 1984	2	4, 20°C	Treated	(31)
Höglund et al., 2002	2	5, 20°C	Combined	(32)
Chung et al., 1993	2	5, 25°C	Natural	(33)
McDaniels et al., 1983	4	8, 26°C	Purified, Treated	(34)

leading to increased die-off (β_1=0.078, SE=0.016, $p < 0.01$). In our model, $\exp(\beta_0)$ is die-off rate at 25°C, and $\exp(\beta_1)$ quantifies the multiplicative change in decay rate per 1°C increase in temperature. Due to the exponential relationship between temperature and die-off, the effect of a given change in temperature was small below 20°C (Figure 1). It is known that competition between microbes can influence decay rates and some of these experiments were conducted in natural water sources that could contain other microbes. To mitigate this concern, we also adjusted for this variable, with natural water sources being the only water type likely to contain other microbes. As expected, experiments conducted in natural water sources (quantified using an indicator variable, χ_{nat}) that could contain other microbes had higher decay rates (β_2=1.21, SE=0.327, $p < 0.01$). Although the effect estimate for temperature attenuated slightly after adjusting for this possible competition effect, the association remained significant. There was no significant interaction between water type and temperature in our dataset.

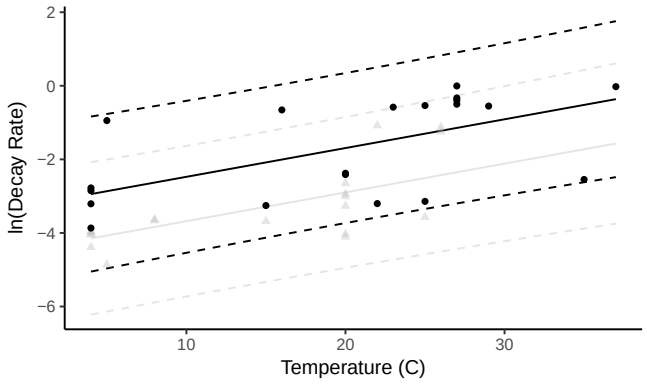

Fig. 1. Temperature (T) and decay rate ($K(T)$) with prediction intervals from the adjusted model. Data, predicted decay rates, and prediction intervals are shown in black for natural water sources and in gray for all other water sources. The predicted decay rates for each temperature are shown in solid lines and the 95% prediction intervals are shown in dashed lines for both types of water sources. For a graph of these decay rates on the linear scale, see supplement.

The adjusted, fitted regression was

$$\log(K(T)) = -2.51 + 0.078(T - 25) + 1.21\chi_{\text{nat}}. \quad [4]$$

For our simulations, we set $\chi_{\text{nat}} = 1$. (Comparisons to simulations run with the model unadjusted for water type may be found in the supplement. The results were similar.)

Amplification in Water Sources. In our simulations, for flowing systems, water can only amplify infection when the available volume of drinking water sources is low, because residence times in the local water supply are too short for pathogen concentrations to accumulate locally. However, for standing water, temperature can be an important determinant of community risk at moderate-to-high volumes, with higher temperatures reducing the strength of indirect transmission (Figure 2) and leading to lower cumulative incidence (Figure 3).

The strength of the temperature effect (adjusted by changing μ) depends on both the strength of direct person-to-person transmission and the size of the water reservoir, with temperature having a stronger effect when direct transmission is weaker (when β_H is smaller) and the per capita water availability is higher (V is higher). When per capita water availability is 1,000 L, temperature only affected risk at higher temperatures, with an average risk difference per °C of 1.0%, 0.3%, and 0% for temperatures between 20°C and 37°C at $\mathcal{R}_{0,H}$ values of 1, 2, and 3 respectively (Figure 3B). When per capita water availability was 10,000 L, temperature had the largest effect on risk at lower temperatures with risk differences of 2.4%, 0.5%, and 0.2% per °C between 10°C and 20°C for $\mathcal{R}_{0,H}$ values of 1, 2, and 3 respectively (Figure 3C). Between 20°C and 37°C, these same risk differences were smaller, with values of 1.9%, 0.3%, and 0.1% respectively.

For standing water at moderate dilution, the amount of sanitation coverage needed to reduce $\mathcal{R}_{0,W}$ below 1 varied by temperature, with higher coverage needed to interrupt waterborne transmission at lower temperatures. When dilution was high, $\mathcal{R}_{0,W}$ was always less than 1 irrespective of the degree of attenuation of shedding. For flowing water, $\mathcal{R}_{0,W}$ was only greater than 1 when dilution was low, and the amount of sanitation coverage needed to reduce $\mathcal{R}_{0,W}$ below 1 depended on the flow velocity, with less coverage needed in faster flowing rivers (Figure 2).

Dissemination through water. In order for an outbreak to move downstream through water sources, we found that $\mathcal{R}_0 > 1$ at the upstream location of outbreak seeding was a sufficient condition for downstream outbreaks, as long as the transmission term from water was positive ($\beta_W > 0$). However, this condition was not necessary when the water was highly concentrated (due to low volume and slow flow rates).

In particular, when direct transmission dominates ($\mathcal{R}_{0,W} \ll \mathcal{R}_{0,H}$), dissemination to the downstream community (i.e. an outbreak at the downstream location) does not occur when the upsteam $\mathcal{R}_0 < 1$, regardless of the initial conditions (Figure 4A). In contrast, when indirect transmission dominates ($\mathcal{R}_{0,H} \ll \mathcal{R}_{0,W}$), outbreaks can occur in the downstream community when the upstream $\mathcal{R}_0 < 1$ if the water volume is low, depending on the initial conditions (Figure 4B). Hence, our model suggests that dissemination of rotavirus along waterways can occur even when local person-to-person transmission conditions are not conducive to outbreaks.

This model behavior is consistent across a wide range of die-off and flow velocity values, including both when there is no direct transmission ($\mathcal{R}_{0,H} = 0$, Figure 5) and when both pathways were active ($\mathcal{R}_{0,H} > 0$ and $\mathcal{R}_{0,W} > 0$ but $\mathcal{R}_{0,H} + \mathcal{R}_{0,W} < 1$). In this last condition, the $\mathcal{R}_0 = 1$ threshold occurs at a higher flow rate whose value is proportional to the

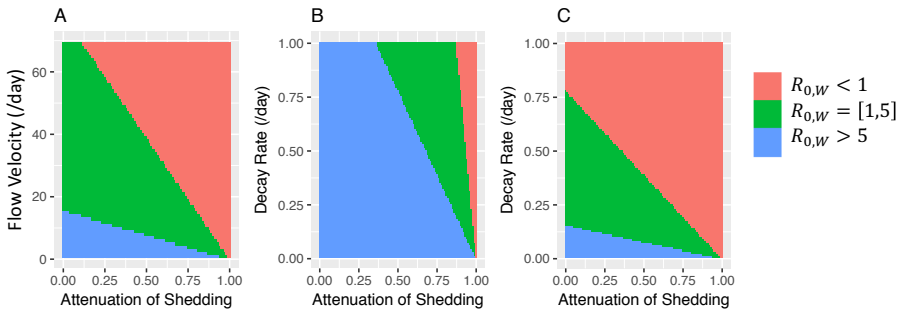

Fig. 2. The strength of indirect, water-human, transmission ($\mathcal{R}_{0,W}$) for A) flowing water ($\nu > 0$) at low volume (10^5 L), B) standing water ($\nu = 0$) at moderate volume (1,000 L/person, 10^6 L total volume), and C) standing water ($\nu = 0$) at moderately-high volume (10,000 L/person, 10^7 L total volume). For flowing water, the flow velocity (ν) is more important than decay rates, so the sensitivity analysis is shown as a function of flow rates (ν) and attenuation due to water, sanitation, and hygiene interventions and natural processes (c). For standing water, the water is not flowing so decay rates (μ) and attenuation of shedding (c) become important for waterborne disease dynamics. For flowing water, the pathogen decay rate was set to 0.056, the median decay rate found in the studies included in our meta-analysis.

strength of direct transmission (Figure 5). At higher water source volumes above 100,000 L (100 L/person), there is no dissemination to the downstream community when the local $\mathcal{R}_0$ is less than 1.

One condition that might prevent dissemination is if rotavirus dies off faster than it is transported. In our simulations, higher die-off rates caused by higher temperature were not sufficient to prevent dissemination through waterways because the die-off rates from our meta-analysis, ranging from 0.008 to 0.996/day (corresponding to mean survival times from 1–125 days), were lower than the river flow rates we observed in our Ecuador field site. Therefore, in practice, the parameter region where die-off rates are higher than flow rates probably only occurs for very slow flowing river systems where flow rates are less than 1 km/day. Furthermore, our simulations suggest that even when flow rates are slower than 1 km/day, dissemination may occur if volume is low.

In summary, our simulations suggest that a sufficient condition for dissemination is that, locally, $\mathcal{R}_0 > 1$ but that there is a narrow parameter regime when the local, upstream $\mathcal{R}_0$ is less than 1 but where dissemination can also occur under low volume conditions.

Discussion

We find that environmental transmission of rotavirus through water sources may be an important source of risk in tropical climates at both the community scale (due to amplification) and regional scale (due to dissemination). The role of the environment in rotavirus transmission was affected by temperature; river flow velocity; water, sanitation and hygiene interventions; and the size of the water reservoir (which determines dilution). Increases in temperature were found to be associated with exponential increases in the decay rate of rotavirus in water sources. We would therefore expect temperature to only appreciably increase die-off in tropical locations where the water temperatures can easily exceed 20°C. However, even at lower temperatures small changes in ambient temperatures can have a larger impact on risk for communities drawing water from more dilute surfaces, such as lakes. Communities linked by flowing rivers could disseminate infection but their water supplies did not amplify within-community outbreaks unless dilution was low, whereas outbreaks in communities with standing water sources could be amplified by environmentally mediated transmission when dilution was low-to-moderate. In the real world, these patterns may help to describe distinct disease dynamics among communities using rivers—as opposed to ponds and lakes—for water sources, as well as communities with riverine water sources with time-varying flow velocity and connectivity. Furthermore, even in communities served by flowing surface water sources, pathogens may accumulate in areas of slow flow along the river's edge (25). Disease dynamics in communities where these zones serve as foci for interactions between the human population and the water source may exhibit qualities of both flowing and standing water communities as described by our model.

Prior researchers have attempted to understand how rotavirus may be influenced by temperature and other seasonal factors using both time series analyses and meta-analysis techniques. In general, these studies have found that rotavirus infections are more common in cooler seasons with average effects of 4–10% decrease in incidence per 1°C increase in temperature (11, 12). In our model, every 1°C increase in temperature leads to a total 0.1–2.4% decrease in incidence for standing water sources, which is at the lower end of the average associations between temperature and rotavirus seen in prior meta-analyses. There are a number of reasons that our results might not conform exactly to the results of these prior time series studies. First, because the relationship between temper-

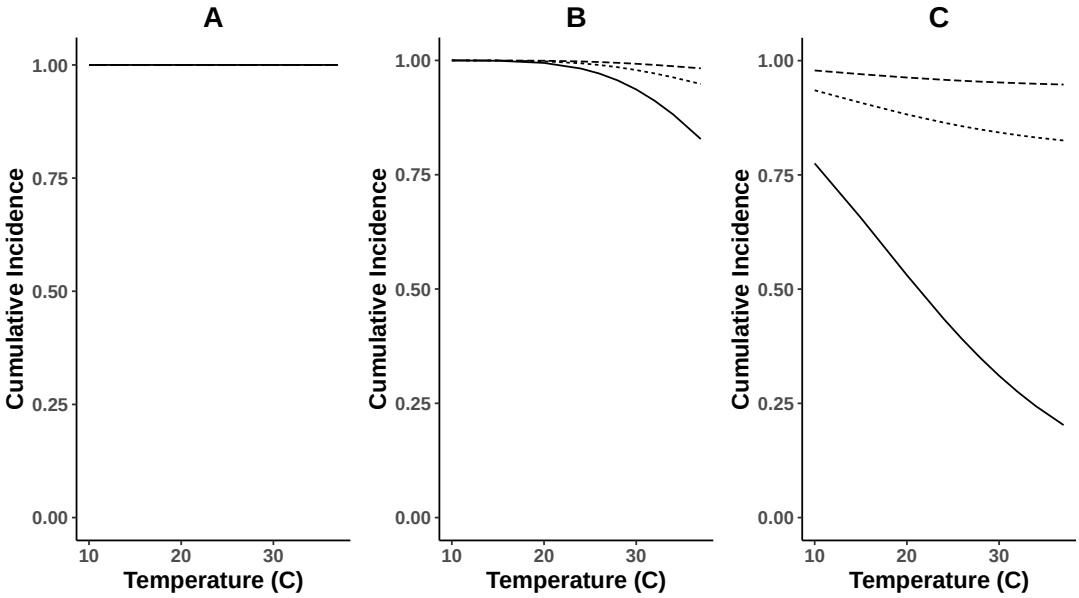

Fig. 3. Cumulative incidence (fraction) for different temperatures and strength of direct transmission. $\mathcal{R}_{0,H}$ values of 1, 2, and 3 are shown in solid, dotted and dashed lines respectively for A) standing water ($\nu = 0$) at low volume (10^5 L), B) standing water ($\nu = 0$) at moderate volume (1,000 L/person, 10^6 L total volume), and C) standing water ($\nu = 0$) at moderately-high volume (10,000 L/person, 10^7 L total volume). For all three panels, attenuation of shedding due to water, sanitation, and hygiene interventions and natural processes (c) is set to 0.9. The maximum effect of temperature occurs at this level of attenuation and high dilution, with $\mathcal{R}_{0,W}$ decreasing from over 0.98 to 0.11 as temperature increases from 10 to 37° C for standing water. For flowing water, temperature has no effect on cumulative incidence for any volume because flow is much faster than decay and $R_{0,W}$ only changes from 0.05 to 0.04 as temperature increases from 10°C to 37°C.

ature and decay is exponential, the effect of any temperature change depends on the baseline temperature. This means that larger changes in pathogen survival are expected in areas that are already hotter on average, such as the tropics. Second, many of the studies considered in these meta-analyses may also be subject to confounding by other unmeasured factors including differences in local hydrological conditions. Thus other seasonal processes are likely to explain much of the remaining temperature association, and the average associations in the literature may be inflated by publication bias (12).

The impact of temperature on disease transmission is likely to be different in temperate compared with tropical climates. In temperate regions, many factors in addition to temperature change seasonally that could cause a large shift in the strength of transmission, including seasonal changes in humidity. Thus, rotavirus seasonality in temperate climates is likely to be multifactorial and the result of many interdependent processes, which we do not explore here. While humidity has a large impact on the decay rate of rotavirus on fomites, temperature has a much smaller impact. The majority of the literature suggests that at high humidity conditions such as those found in the tropics, rotavirus survival on surfaces is likely to be long enough to sustain transmission for all temperatures (14–18). Thus, while direct transmission may have a seasonal component, particularly in temperate climates, it is unlikely that the seasonality of rotavirus transmission in the tropics can be explained by temperature-related variations in fomite-mediated transmission alone (14–18). A detailed analysis of seasonal changes in transmission parameters for both pathways is beyond the scope of this paper, but our results do suggest that temperature, and its impact on waterborne transmission, might impact seasonality in rotavirus transmission, particularly in the tropics.

Although our meta-analysis showed that higher temperatures were associated with faster decay, our model predicts that this effect of temperature would not be sufficient to prevent dissemination through water sources in sites similar to our study site in Ecuador. For communities with standing water sources, water also acted as an amplifier of local transmission. Even though the degree of coupling of infectious pathogens between the water supply and infectious people is probably lower in large cities that use standing water sources, the sensitivity of our model to the per capita water availability suggests that larger cities with poor sanitation infrastructure where most residents get their drinking water from a single reservoir may be especially vulnerable to waterborne transmission. For communities using standing water sources, the effect of temperature was more pronounced, and higher temperatures led to smaller outbreaks due to the decreased persistence of rotavirus in the water supply.

Dissemination of rotavirus through flowing waterways. Together, our results suggest that water is likely to be a sufficient disseminator of infection in most flowing systems. Although it is not necessary for the overall reproduction number in the upstream community to be greater than 1 for dissemination to occur, in most contexts the sufficient condition ($\mathcal{R}_0 > 1$) is likely to be met either through direct transmission alone ($\mathcal{R}_{0,H} > 1$) or indirect transmission if there is increased contamination levels due to low water volumes ($\mathcal{R}_{0,W} > 1$). Because the overall reproduction number is a function of both direct and indirect transmission, it is sufficient for the sum of both submodel $\mathcal{R}_0$'s to be greater than 1. The submodel reproduction number $\mathcal{R}_{0,H}$ is likely to be greater than 1 in most contexts in the absence of focused interventions targeting this pathway, both because the shedding rate of rotavirus

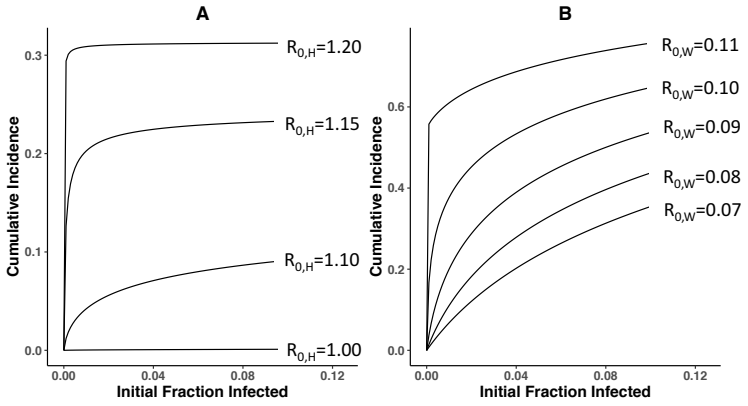

Fig. 4. The effect of increasing the initial upstream outbreak size on cumulative incidence in the downstream community when A) Most transmission occurs directly from human to human and the submodel reproduction number for water was <0.01, and B) All transmission occurs through the water and direct transmission is set to 0. This result only occurs at low volumes (100,000 L).

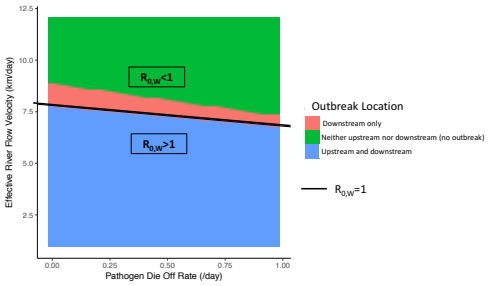

Fig. 5. Location of outbreak (downstream and/or upstream community) as a function of pathogen decay rates and effective flow velocity. An outbreak is defined by a cumulative incidence of at least 5% after 365 days. The solid line corresponds to the situation when $\mathcal{R}_{0,W} = 1$. Above this line, $\mathcal{R}_{0,W} < 1$ and below this line $\mathcal{R}_{0,W} > 1$. For these simulations, the sub-model reproduction number for direct transmission was set to 0 and volume was set to 100,000 L.

is extremely high relative to its median infectious dose and because rotavirus is persistent on a variety of environmental surfaces (14–18). When $\mathcal{R}_{0,H} > 1$, the sufficient condition for dissemination will always be met, as long as water remains sufficient to disseminate enough pathogens to cause an outbreak in downstream communities. Even when direct transmission is not sufficiently high, low water volumes (increasing indirect transmission) or high initial outbreak sizes (increasing the probability of a breakthrough transmission event) may allow water to disseminate infection between communities.

Multiple pathways: direct and indirect transmission. While some seasonality patterns in rotavirus incidence may be partially explained by dynamic resonance related to the birth rate, many other factors may underlie this typical seasonal pattern of risk (8, 9, 35), including environmental variables like temperature. Because rotavirus is highly infectious, direct transmission alone may be sufficient to cause nearly universal infection of the susceptible population. However, in this analysis we have identified situations in which indirect transmission can affect the spread of disease through a watershed and may also be sufficient to cause large outbreaks. When two sufficient causes (i.e., environmental and direct transmission) are both met in a population(36), conventional epidemiologic studies that analyze case data alone may not detect this environmental transmission because multiple mechanisms are capable of explaining the observed dynamics (2, 6, 37, 38). By explicitly considering this environmental route of transmission, we were able to better explore the potential importance of both pathways. Our model shows that while direct human-to-human transmission is likely to be more important for local amplification, indirect transmission may be sufficient to both sustain and spread outbreaks. Furthermore, if one assumes uninterrupted flow and surface water availability, waterborne transmission appears to be more important in cooler and drier seasons, when rotavirus persistence in the environment is high

and the dilution effect is weak. In settings where certain assumptions of our model hold, i.e., that accumulation of and exposure to waterborne rotavirus can be adequately represented with a single water compartment and where access to safe water and sanitation is poor, inland cities with poor sanitation access are also expected to be especially vulnerable to waterborne transmission because of their relatively lower per capita water availability. In these settings, interventions like vaccination that impact both direct transmission and indirect pathways may be needed to reduce risk of infection within communities and to stop the spread of infection through watersheds.

Our model shows that the degree of intervention needed to interrupt water transmission depends on both local hydrologic conditions and ambient temperature. For standing water systems, less effort may need to be invested to prevent the accumulation of viruses in local water bodies when ambient temperature is high. For communities that utilize flowing surface water systems, targeting direct transmission alone may be sufficient to prevent disease spread as long as the size of the water reservoir is relatively large. When the size of the water reservoir is relatively small, the degree of intervention needed in flowing systems depends on the speed of water flow. Furthermore, if water is in fact an effective disseminator of rotavirus, restricting person-to-person contact may be sufficient to prevent the spread of rotavirus between communities for systems with greater per-capita water availability. In other types of communities, direct human-to-human transmission may be important for local amplification, but indirect transmission may sustain and spread outbreaks. This information can help public health practitioners identify optimal approaches to decrease environmentally mediated rotavirus risks in resource-limited settings by considering local climatological and hydrological conditions.

We acknowledge that many questions remain about how rotavirus is transmitted: there is strong evidence both that the overall reproduction number $\mathcal{R}_0$ is high and that the environment can be important for transmission. In fact, the processes underlying rotavirus transmission are likely to be highly complex, with the potential for super-spreading and large heterogeneities in transmission by age (6). In the United States, where drinking water and sanitation facilities are generally safe, rotavirus was still a major source of disease risk prior to the introduction of the vaccine, suggesting that water and sanitation interventions alone are unlikely to be adequate to interrupt transmission (39). Because water remained an important source of risk in our model for communities resembling larger cities even when shedding was highly attenuated, our results are consistent with this line of research. Future work combining environmental sampling over multiple seasons with case data would be particularly useful to help validate our conclusions. Genetic data could also help clarify the relative importance of different transmission routes. In addition, future studies on the effect of humidity on rotavirus persistence on fomites could help clarify other sources of seasonality in direct transmission.

Implications for trends in rotavirus risk under climate change. Given that global temperatures are projected to increase as climate change proceeds (40), many researchers have attempted to understand the relationship between temperature and risk of disease. In our model, we showed that one reason for the observed decrease in rotavirus infections at higher temperatures might be increased die-off of rotavirus in water sources. Other pathogens, however, have different temperature signatures, and many studies have shown that risk of bacterial and protozoan diarrhea is, in fact, slightly elevated at higher temperatures (12). However, these associations do not necessarily preclude increased die-off at higher temperatures for bacteria, but rather reflect the net balance of factors for bacteria compared with viral pathogens. For example, for many bacterial species, temperature activated virulence genes have been identified which may lead to increased risk of disease at higher temperatures even in the presence of faster die off (21, 22). Some bacteria may also have higher replication rates at higher temperatures (12). One implication, then, of our results is that the etiology of waterborne diarrhea cases might shift away from rotavirus and towards bacterial and protozoal infections as global mean temperatures continue to increase. On the other hand, rotavirus may be able to adapt to slowly increasing temperature, suggesting the need to study the temperature sensitivity of rotaviruses from different climates (41). Another potential implication is that the waterborne transmission route for rotavirus may become less important as the average temperature increases, even if overall transmission does not decline. Given the non-linear relationship between temperature and die-off, this shift is more likely in the tropics.

Conclusions. While some researchers acknowledge that waterborne transmission of rotavirus can occur, its importance is often downplayed relative to direct transmission (5). Our model analysis reaffirms that direct transmission likely plays a central role in amplifying rotavirus infection within communities. We used a transmission model parameterized using temperature-specific rotavirus die-off data to show that water is sufficient to both disseminate rotavirus infection between hydrologically connected communities, initiating local transmission cycles, and to amplify transmission under conditions of relative water scarcity in flowing systems and in standing water for a wide range of water availability. Our model indicates these environmental effects are most important in cooler seasons and, may be particularly important in larger cities with poor sanitation. These findings highlight the need to consider waterborne pathways in rotavirus control interventions, particularly in endemic regions in the tropics where the accumulation of virus in waterways may be more pronounced.

Materials and Methods

An overall diagram of our conceptual framework and the flow of analysis is shown in Figure 6 below (adapted from (19)). We built an ordinary differential equation (ODE) transmission model to examine the role of temperature on rotavirus transmission patterns. To parameterize this model and to assess the effect of temperature on this waterborne pathway, we conducted a meta-analysis of studies that provided data on rotavirus die-off rates in water and developed a statistical model relating temperature to die-off rate. We then used this model to assess the role of water in dissemination of rotavirus between communities and amplification of rotavirus infection cycles within communities. All analyses were conducted in R (v. 3.1.2). We integrated the ODE model using the `deSolve` package.

Transmission Model Structure. To explore the potential for hydrological transmission of rotavirus infections between sites, as well as the climate sensitivity of such an epidemiological system, we developed a susceptible, infectious, recovered (SIR) model representing two hydrologically-connected communities at either end of

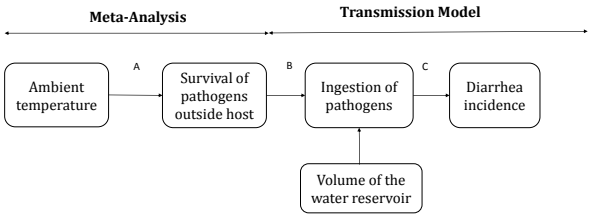

Fig. 6. Conceptual framework of the analysis. The meta-analysis portion of our study informs arrow A, whereas the transmission model analysis informs arrows B and C. The transmission model also considered the role of the size of the water reservoir, which may vary based on the type of water source a community uses. This figure was adapted from (19).

a 10 km river reach. In our model, susceptible, infectious, and recovered individuals in community i are given by compartments S_i, I_i, and R_i, and transmission can be either direct—from contacts between susceptible and infectious persons, which occur at rate β_H—or indirect—from ingestion of pathogens persisting in the aqueous environment (with contact rate β_W and per contact volume ingested ρ). The water compartments (W), described in more detail below, model the concentration of pathogens in the water. We assume that individuals within communities draw water from only one water source, such that the concentration in the water compartment is a reasonable proxy for exposure for all individuals within that community. We do not model human movement between communities.

We assume that infectious individuals shed pathogens into the environment at daily rate ϕ, but that the number of shed pathogens is attenuated prior to entering the water compartment by a combination of natural environmental decay and local sanitation and hygiene interventions (removing a fraction of pathogens c). In communities with built infrastructure, this parameter c reflects not only processes relating to runoff and decay in the environment, but also processes related to wastewater treatment, and thus most likely has a higher value. Larger cities may also have higher levels of attenuation due to their more complex topology. Upon deposition in the water, pathogens are diluted based on the total volume of the community's water source (V_i). Prior researchers have suggested that the per capita volume of water resources available may be smaller for large settlements with built infrastructure than for smaller communities, like those modeled here, where the size of the water reservoir is determined by natural rainfall and surface water flows, which often leads to greater dilution of shed pathogens (42). We thus simulated across a range of available volumes by adjusting to produce water reservoir sizes that were consistent with a small pond (low dilution) to a medium-sized lake (high dilution) (43) (see supplement for additional details). The low dilution scenario also approximates the minimum the per capita rate of water availability targeted for larger cities with piped water (44). Using our meta-analysis (procedures described below), we assume that the concentration of rotavirus in the water attenuates as a first order reaction with rate $\mu = K(T)$ (eq. (Eq. (3)). We model infections produced by ingestion of contaminated water using a linear dose–response function (45), which we deemed adequate because the pathogen doses in our simulations fell within the linear range of the typical dose–response curves (46).

We modeled movement of rotavirus from upstream to downstream communities using a mixing cell hydrological transport model (47). In brief, this model approximates the advection–diffusion equation for solute transport, modeling downstream flow through a series of compartments representing river reaches. While no diffusion of pathogens occurs in the upstream direction from point source inputs, the method produces a moving distribution of pathogen concentrations, with mean position at time after entering the system determined by the velocity of advection, and variance in pathogen position at time , i.e. rate of diffusion, proportional to the product of the advection velocity and cell length(47). Thus, flowing systems and reasonably large spatial scales (i.e. where little diffusion in the upstream direction of pathogen sources is expected), our mixing cell approach closely approximates a solution to the advection–diffusion equation. In our model, we used a total of 11 compartments (one for each village and nine intermediary), each representing a 1 km stretch of river, between which the rate of flow is determined by the flow velocity, which is given by ν. We assume that there is no sedimentation of pathogen between communities given the small virus particle size and the length of the river reach between communities. However, attenuation due to sedimentation could be approximated by assuming a slightly lower flow velocity. Pathogens entering the water at the upstream community are allowed to either move to the next compartment or decay, and no pathogens can be shed into the water between villages. More details about the mixing cell model, including comparisons with other methods, is in the supplement.

The model diagram is shown in Figure 7, and the model equations are given below. Parameters used in simulations and their values are shown in Table 2.

$$
\begin{aligned}
\dot{S}_i &= -\frac{\beta_H}{N} S_i I_i - \frac{\beta_W \rho}{2} S_i W_i, \\
\dot{I}_i &= \frac{\beta_H}{N} S_i I_i + \frac{\beta_W \rho}{2} S_i W_i - \gamma I_i \\
\dot{R}_i &= \gamma I_i, \\
\dot{W}_1 &= \frac{(1-c)\phi}{V_1} I_i - (\mu + \nu) W_1, \\
\dot{W}_0^{\text{int}} &= W_1, \\
\dot{W}_j^{\text{int}} &= \nu W_{j-1}^{\text{int}} - (\mu + \nu) W_j^{\text{int}}, \qquad 1 \le j \le 9, \\
\dot{W}_2 &= \frac{(1-c)\phi}{V_2} I_2 + \nu W_9^{\text{int}} - (\mu + \nu) W_2,
\end{aligned}
\qquad [5]
$$

The transmission rate parameters β_W and β_H and the recovery parameter γ are assumed to be the same for both communities. In this simulation, the two communities have the same population size and available water volume. Flow velocities between the two sites are reasonable approximations of the hydrological characteristics of our study site in rural, coastal Ecuador (24), which encompasses three rivers with distinct average flow velocities: the Onzole (17 km/day), the Cayapas (26 km/day), and the Santiago (69 km/day) (25).

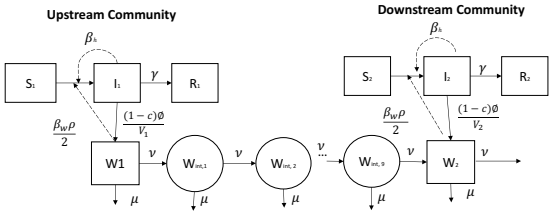

Fig. 7. Rotavirus transmission model diagram. Dashed arrows represent transmission events and solid arrows represent transition of people or pathogens between model compartments. Villagers and city residents are classified as susceptible (S), infected (I) and Recovered (R). We also track contamination in the village water source (W). Susceptible people can become infected by either direct person to person transmission or indirect person-water-person transmission within their own community. Pathogens in the local water source can travel from the upstream to the downstream community through the water source. Pathogens in water sources can also decay in each community or between communities. The model parameters are defined in Table 2.

Table 2. Parameter estimates, units, and their sources. Water volume calculations and details regarding the scaling of flow velocity are shown in the supplement.

Parameter	Symbol	Units	Value	Source
Transmission rate from human to human	β_H	1/day	[0, 0.6]	Varied
Transmission rate from water to human	β_W	water contact/day	3	(48)
Recovery rate	γ	1/day	0.2	(49)
Median infectious dose	n_{50}	ffu	10	(7)
Shedding rate	ϕ	n_{50}/person/day	2,605	(7, 50–52)
Attenuation of shedding	c	–	[0, 1]	Varied
Water consumed per day	ρ	volume/water contact	0.4	(48)
Population size	N	people	1000	Assumption
Water volume	V_i	L	$[10^5, 10^9]$	Varied
Pathogen decay rate	μ	1/day	[0,1]	$K(T)$
Flow velocity	ν	km/day	[0, 69]	(25)

Literature Review and Meta-Analysis.

Data Collection. To parameterize our transmission model, we retrieved a comprehensive list of studies that estimate rotavirus die-off in water samples at known temperatures by querying PubMed, Scopus, and Web of Science with the following search string:

(rotavirus OR 'rotavira') AND (temperature OR heat) AND ('die off' OR inactivation OR dieoff OR growth OR survival) AND (water OR aqueous OR aquatic OR marine).*

Studies were included that: 1) reported results from at least one rotavirus experiment; 2) that took place in purified or treated water (i.e., reverse osmosis water, sterile distilled water, tap water, etc.), natural water (e.g., from lakes, rivers, creeks, etc.), post-treatment (i.e., tap water, filtered water, etc.), or simple aqueous solutions at neutral pH (i.e., phosphate-buffered saline (PBS)); 3) reported a steady water temperature that did not fluctuate more than 5°C during the course of the experiment; and 4) provided a clear explanation of methods used to assay rotavirus concentration over time. Studies conducted in more complex media (e.g., that added natural organic matter, humid sludges, etc. to aqueous media) were examined for control experiments conducted in pure water but were otherwise excluded. Also excluded were studies that reported only the time required for total inactivation of the rotavirus population.

Temperatures reported in die-off experiments were recorded directly from reviewed studies. Where mean rotavirus concentration was available in a figure or plot, data were extracted using DataThief software (53). Additional information regarding rotavirus serotypes, water source, water treatment, initial viral concentration was also recorded.

Statistical Analysis. We determined the relationship between temperature and die-off rates in two steps. First, we estimated the die-off rate for each experiment. Second, we estimated the relationship between die-off rates in each study and the corresponding temperatures.

The rate of change in viral concentration is commonly modeled as a simple first-order decay process where $N(t)$ is the pathogen population at time t; N_0 is the initial population; and K is the first-order rate constant (54):

$$N(t) = N_0 \times e^{-Kt} \quad [6]$$

We used this framework to model pathogen decay in our analysis. We also examined the data for evidence of tailing off, or biphasic decay (55), but few experiments appeared to exhibit biphasic decay and those that did lacked sufficient observations for us to estimate the parameters. We therefore assumed that the true pattern of decay was monophasic for all studies.

After obtaining an estimate of K in each experiment, we calculated the relationship between temperature and the decay rate of rotavirus in aqueous environments. We modeled the decay rate K of rotavirus as a non-linear function of temperature (T) (Eq. (3)) and fit this model using generalized least squares, following prior published approaches used for other pathogens.

Transmission Model Analysis. All model simulations were seeded with one infectious case in the upstream community and were run for 365 days. Both communities were assumed to have a population of 1,000. To better understand the threshold and equilibrium features of our model, we calculated the basic reproduction number, $\mathcal{R}_0$, for the local communities using the next generation method (56). Briefly, $\mathcal{R}_0$ captures the expected number of secondary cases produced by one case during its infectious period after being introduced into a completely susceptible population. If this number is greater than 1 (e.g., greater than replacement), an outbreak seeded near the disease-free equilibrium will transiently grow, and, if it is less than 1, such an outbreak will die out.

The spatially local $\mathcal{R}_0$ we derive does not necessarily control the outbreak dynamics on the whole system, nor does it precisely control whether an outbreak will occur in the downstream community if seeded in the upstream community. In a similar model, Gatto et al showed that for pathogen invasion to occur along a network it is necessary that the basic reproduction number for at least one of the communities in the network be greater than 1 (57). Hence, we use the spatially local $\mathcal{R}_0$ for the upstream community as a dynamically relevant and intuitive benchmark for our simulation studies.

Amplification. To determine if water could amplify infection by changing the size of the outbreak, we estimated the overall attack ratio, that is, the cumulative incidence, as a function of the strength of each pathway and estimated how cumulative incidence changed as temperature changes impacted rotavirus decay rates. We also assessed how local hydrologic conditions and attenuation of shedding might impact risk (defined by cumulative incidence at the end of the simulation) both overall and from water sources alone.

Dissemination. We defined dissemination as 5% of the population in the downstream community being infected by the end of the simulation (365 days). We investigated how dissemination might depend on temperature, river flow velocity, and dilution for biologically plausible parameter values (see table 2 for credible parameter ranges). Because sub-threshold outbreaks can occur even when $\mathcal{R}_0 < 1$ (56) and knowing the parameters is alone is not always sufficient to predict whether or not an outbreak will occur, we also analyzed whether or not downstream outbreaks occurred in our model for biologically plausible changes in initial conditions (initial outbreak sizes up to 10%).

We recognize that human travel is also an important disseminator for rotavirus and is needed to explain spread of disease to upstream communities that would not otherwise become infected through flowing waterways. However, in this analysis, we aimed to determine if water was also a sufficient disseminator to downstream communities. Thus, we acknowledge that waterborne dissemination is not necessary to spread disease between communities but aimed to determine if it was also a sufficient pathway.

For both dissemination and amplification analyses, we used the fitted model equation from the meta-analysis to calculate decay rates at different temperatures, simulating across the range of decay rates seen in the literature.

ACKNOWLEDGMENTS. A.N.M.K., N.L.,A.F.B., and J.N.S.E. were supported by the National Institutes of Health (grant R01-AI050038) and NIH MIDAS (grant U01GM110712). P.A.C. and J.V.R. were supported by National Institutes of Health (grant R01TW010286, R01AI125842 and K01AI091864), by the National Science Foundation Water, Sustainability and Climate (grants 1360330 and 1646708), and by the University of California Multi-campus Research Programs and Initiatives award MRP-17-446315.

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