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Quantification of Human Norovirus GII on Hands of Mothers with Children under the Age of Five Years in Bagamoyo, Tanzania

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Abstract. Human noroviruses are the most common cause of viral gastroenteritis worldwide and one of the leading causes of viral diarrhea in children under the age of 5 years. Hands have been shown to play an important role in norovirus transmission. Norovirus outbreaks tend to exhibit strong seasonality, most often occurring during cold, dry months, but recently have also been documented during hot, dry winter months in the southern hemisphere. Other research suggests that rainfall is an important factor in norovirus outbreaks. This study examines the prevalence and concentration of human norovirus GII on the hands of mothers in Bagamoyo, Tanzania, during the rainy and dry seasons. Norovirus GII was detected in approximately 5% of hand rinse samples during both the rainy and dry seasons. Fecal indicator bacteria levels, *Escherichia coli* and enterococci, in hand rinse samples were not associated with norovirus hand contamination. Turbidity of the hand rinses was found to be associated with norovirus presence on mothers' hands; however, this relationship was only observed during the rainy season. The results suggest mothers' hands serve as a source of norovirus exposure for young children in Tanzanian households, and further work is needed to determine better indicators of norovirus contamination in these environments.

INTRODUCTION

Over half a million children under the age of 5 years die every year from diarrheal diseases.[1] Human noroviruses are the most common cause of viral gastroenteritis worldwide[2] and one of the leading causes of viral diarrhea in children under the age of five years.[3] In addition, recent studies have shown noroviruses to be associated with childhood diarrhea in low-income countries such as Tanzania,[4,5] where diarrhea is one of the most common causes of under-five mortality.[6]

Noroviruses are single-stranded RNA, non-enveloped viruses belonging to the family Caliciviridae.[7] Human noroviruses are represented by three genogroups (GI, GII, and GIV)[7]; though the majority of reported outbreaks and cases are caused by GII.[2] Noroviruses are transmitted via the fecal-oral route, as well as through vomitus.[2] People can become infected with norovirus through consumption of contaminated food or water, or through direct contact with contaminated fomites or hands.[8] Although the relative contribution of these transmission routes to the burden of norovirus illnesses is not known, recent studies have shown that hands are often contaminated during periods of infection and can act as vehicles for norovirus transmission long after the initial contamination event has occurred.[9,10] The importance of hands is consistent with the fact that noroviruses are considered to be the primary causative agent of food-borne disease outbreaks,[11] and the contamination source for the majority of those outbreaks can be traced back to infected food handlers.

Norovirus outbreaks have been long reported to exhibit strong seasonality, most often occurring during cold, dry months.[7] However, the majority of the data supporting this seasonal trend originates from studies conducted in the temperate climates of North America and Europe.[12] Moreover, recent research has shown that by contrast, outbreaks in the southern hemisphere tend to occur in warmer months.[13,14] In addition, one study found a strong association between rainfall and norovirus outbreaks in Australia.[15] This suggests that rainfall may also be an important factor in norovirus outbreaks.

Despite the importance of norovirus in childhood diarrhea and the role that hands are believed to play in norovirus transmission, there are currently no data on norovirus contamination of hands in low-income countries. The goal of this study is to determine the prevalence and concentration of norovirus GII (NVGII) on the hands of mothers with children under the age of five years in Bagamoyo, Tanzania, during both the dry and rainy seasons.

MATERIALS AND METHODS

Setting. Samples analyzed for this study were collected from households located in the Bagamoyo District of Tanzania, east Africa (6°W28′ S 38°W55′ E). All households enrolled in the study included at least one child under 5 years of age. Households were defined as groups of people that sleep and eat together in a dwelling on a regular basis. The hand rinse samples analyzed for this study were collected from a subset of 1,219 households surveyed during a household water and hygiene behavioral intervention trial. The hygiene arm of the behavioral intervention examined whether the provision of personal microbiological hand contamination data (presence/absence of enterococci) affected a mother's hygiene behaviors such as hand washing frequency. Samples used in this study were collected from households assigned to the control and hygiene intervention cohorts during the baseline visits that occurred from March–May (rainy season) 2010, and from the same households during the first follow-up visit after intervention, which occurred from September–October (dry season) 2010.[16] These households were unique from those previously described.[17,18]

Households were chosen for this study by randomly selecting from household clusters in the larger, behavioral intervention trial. In brief, clusters were determined from Geographic Information Systems data collected for each of the 1,219 households. Because of the lack of sample availability,

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households whose hand rinses were processed in previous studies[17,18] were excluded from selection for this study. Therefore, because one of the previous studies[17] evaluated all of the households from the baseline visit with a child under the age of five years self-reporting (by his or her caretaker) symptoms of highly credible gastrointestinal illness (HCGI), there were no households in this study containing children under the age of five years with reported HCGI at baseline (rainy season sampling). HCGI was defined as having three or more loose, watery stools within a 24-hour period, blood in the stool, and/or vomiting using a 2-day recall period.[19,20]

The remaining eligible households comprised of 51 spatially coherent household clusters with a mean cluster size of 10 households. A random number was then assigned to each household and sorted in ascending order, first by the random number and then by cluster number. Starting from the smallest random number value, one household was selected from each cluster until a sample size of 88 was reached. Between one and three households from each cluster were selected. As the prevalence of NVGII on hands of mothers in Tanzania is unknown, the sample size was determined by resources and available hand rinse samples archived during the larger behavioral intervention study conducted in 2010 rather than a power analysis.

Data collection. In each participating household, local enumerators collected a hand rinse sample from the adult female caregiver/head of household. Hand rinse samples were collected from the same respondent during the rainy and dry season visit. In brief, hand rinse sampling involved the participant placing her hands, one at a time, into a sterile sample bag containing 350 mL sterile distilled water. This sampling method has been used successfully in a number of previous studies.[18,21–23] Before taking the hand rinse sample, the enumerator recorded the length of time since the female head of household reported she had last washed her hands and noted whether dirt was visible on her palms or underneath her fingernails. Self-reported health data were also collected from the respondent on herself and each child under the age of five years. The respondent reported whether any of the following symptoms presented during the previous 2 days: abdominal pain, three or more loose watery stools within a 24-hour period, blood in their stool, nausea, vomiting, fever, constant coughing, congestion, or difficulty breathing.

Enumerators also conducted interviews with each respondent, asking questions about water consumption, household water and sanitation services, hand hygiene behavior, as well as household demographics. Enumerators participated in a 4-week training, which included instruction on survey content, electronic data collection, sterile sampling technique, and extensive practice and pretesting in non-enrolled households.

Laboratory analysis. All hand rinse samples were stored in a cooler on ice and transported to a local laboratory for microbial analysis by membrane filtration within 6 hours of collection. Turbidity of the samples was measured using a LaMotte 2020e/i Turbidity Meter (LaMotte Company, Chestertown, MD). Fecal indicator bacteria (FIB), *Escherichia coli* and enterococci, were enumerated using membrane filtration following U.S. Environmental Protection Agency Methods 1604 and 1600, respectively.[24,25] Volumes of 1 and 10 mL were processed for culturable FIB analyses, and the range of quantification was 35–175,000 colony forming unit (CFU) per two hands.

Hand rinse samples were processed for norovirus also by membrane filtration through 47-mm, 0.45-μm pore size nitrocellulose filters (HA type filters; Millipore, Billerica, MA). Before filtering, 0.5 mL of 2.5 M $MgCl_2$ (JT Baker, Hot Springs, AR) was added to every 50 mL water sample to facilitate capture of viral particles.[26] The volume filtered for virus detection in hand rinse samples was 100 mL. Three separate samples were filtered for 25 mL, 37 mL, and 50 mL, respectively, because of their extremely high turbidity. Extraction blanks and duplicates were run daily. After filtration, virus filters were treated with RNAlater (Qiagen, Germantown, MD) to stabilize RNA/DNA[27] and stored at −80°C. Filters were stored for up to 5 months at −80°C until being transported back to Stanford University (Stanford, CA) for molecular processing.

Molecular processing. Total RNA and DNA were extracted simultaneously from the filters using a modified MoBio PowerWater® RNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA) (see Mattioli and others[18] for further details on extraction).[17,28,29] Separate 25 μL aliquots of extracted nucleic acids were stored at −80°C for subsequent molecular analyses. RNA/DNA aliquots underwent a maximum of one freeze-thaw cycle before molecular analysis.

NVGII was enumerated in hand rinse samples according to a modified version of the methods described in the work of Viau and others.[28] NVGII was chosen for the study as it is reportedly responsible for most documented outbreaks.[2] In brief, reverse transcriptase quantitative polymerase chain reactions (RT-qPCRs) were prepared using AgPath-ID™ One-Step RT-PCR Kit (Ambion, Grand Island, NY). A total reaction volume of 25 μL was prepared consisting of 6 μL RNA template, 1× Ag PathID Buffer, 1× Ag PathID Enzyme Mix, and 200 nM of each primer and probe (Table 1). All samples were run in triplicate reactions on 96-well plates (Applied Biosystems [ABI], Carlsbad, CA) on an Applied Biosystems StepOnePlus™ thermocycler (ABI). Cycling parameters included a 30-minute reverse transcription step at 50°C, followed by a 10 minute denaturation step at 95°C and then 45 cycles of 95°C for 15 seconds and 60°C for 60 seconds.

A synthetic single-stranded RNA standard, Synthetic Norovirus G2 (II) RNA (ATCC® VR3200SD™), was acquired from American Type Culture Collection (ATCC, Manassas, VA). Ten-fold serial dilutions were made of the synthetic RNA to create standards that ranged from 3×10^0 to 3×10^5 copies per 6 μL of standard. Standard dilution series were run in triplicate with each 96-well plate run of samples, and the standard curve was used to determine the target copy number in each unknown sample, as well as the efficiency of the assay (Table 1).[32] The amplification threshold was set to 0.02 ΔRn units, and a sample was considered positive if amplification occurred before a threshold cycle (Ct) of 44. Triplicate no template controls (NTCs) and extraction blanks were also included in every run. The presence of inhibition was assessed in 28 randomly selected hand rinses RNA extracts that were negative for norovirus. RNA extracts were spiked with 10^3 copies of the RNA target and their Ct was compared with the Ct of a standard reaction containing only 10^3 copies of the RNA target.

Copy number estimates of NVGII per reaction were made for all samples, even those with a Ct above that of the lowest concentration standard. For the latter, copy number estimates were obtained by extrapolating from the linear fit of

TABLE 1
Quantitative RT-PCR NVGII assay and pooled standard curves

Target	Gene	Primer/probe*	Primer/probe sequence (5′ to 3′)†	Primer/probe ref	Product size (nucleotide)	Annealing temperature	Pooled curve slope, intercept (R^2)‡	qPCR efficiency
NVGII	OFR1-OFR2 junction	QNIF2d	ATGTTCAGRTGGATGAGRTTCTCWGA	30	88	60°C	−3.313, 37.603 (99.5%)	100.36%
		COG2R	TCGACGCCATCTTCATTCACA	31				
		QNIFSP	FAM-AGCACGTGGGAGGGCGATCG-TAMRA	31				

RT-PCR = reverse transcriptase polymerase chain reactions; NVGII = norovirus GII.
*F denotes sense primer, R denotes antisense primer, and P is hydrolysis probe sequence.
†Mixed bases in degenerate primers and probe are as follows: R, A, or G; W, A, or T. The TaqMan probe was labeled at the 5′ end with the reporter dye FAM (6-carboxyfluorescein) and at the 3′ end with the quencher dye TAMRA (6-carboxytetramethyl-rhodamine).
‡Concentrations were determined with the following formula: Ct = slope × $\log_{10}$ (concentration) + y-intercept. PCR efficiency = $10^{-1/\text{slope}} + 1$.

the log-transformed standard copy number versus Ct (Table 1). The number of gene copies in all three sample replicates were summed and normalized by the virtual volume of hand rinse in the three reactions and then multiplied by the volume of the hand rinse (350 mL) to estimate a density in units of gene copies per two hands.[28] Based on a filtration volume of 100 mL of hand rinse water, the lowest detectable concentration of NVGII was 24.3 copies per two hands (assuming amplification of one target copy in at least one of the triplicate RT-qPCR per sample).

Data analysis. Data were analyzed using SAS Enterprise Guide version 4.3 (SAS Institute Inc., Cary, NC). NVGII copy number per two hands, FIB, and turbidity values were $\log_{10}$ transformed. If FIB were lower than the limit of detection, half the detection limit was used. If FIB were too numerous to count (> 500 CFU per filter), the concentration of bacteria in the sample was calculated assuming 500 CFU per volume filtered. χ^2 or Fisher's exact tests (FET) were used to analyze associations and differences of proportions between binary variables. *t* tests were used to test differences of means between continuous variables. Pooled variances were used unless the Equality of Variances Folded F-Statistic was significant ($P < 0.05$), in which case a Satterthwaite test of unequal variance was used. Pearson correlation coefficients (r_p) were used to evaluate the linear relationship between concentration measurements. Results are considered statistically significant at a level of $P \leq 0.05$.

Ethics statement. Participants were informed in the local language (Kiswahili) of all study procedures and the time required for participation. Written informed consent was obtained from the mother or primary female adult caretaker. The Tanzanian Commission for Science and Technology, the Tanzanian National Institute for Medical Research (NIMRI) Ethics Sub-Committee, the Ifakara Health Institute Institutional Review Board (IRB), and Stanford University's IRB (IRB protocol no. 17971) approved the consent procedures and study protocol.

RESULTS

Household demographics. The age of the mothers and primary caregivers (hereafter referred to collectively as "mothers") surveyed ranged from 17 to 75 years. Of mothers, 78% were able to read and write, and 24% worked outside of the home. On average, households in the study had one child under the age of five years, and 17% had at least one infant (< 1 year old) present. Sixty-six percent of homes were located within Bagamoyo Town, although only 27% of households had electricity. Fifty-six percent of households had a private toilet, and 83% of households reported collecting their stored drinking water from an improved source (tap, borewell, or rainwater).[33]

Hygiene behaviors. During the rainy season sampling (March through May 2010), mothers reported washing their hands with soap an average of 3.0 times (standard deviation [SD] = 2.3, range = 0–10) the day before the visit and using 2 L (SD = 1.8, range = 0–15) per capita each day for hand washing. Twenty-two (28%) mothers reported hand washing within 1 hour before hand rinse sampling; 12 (14%) had visible dirt on the palms; and 48 (55%) had visible dirt beneath their nails. At the dry season visit (September through October 2010), mothers reported washing their hands with soap an average of 3.7 (SD = 1.8, range = 1–7) times the day before the visit and using 1.8 L (SD = 0.8, range = 0.5–5) per capita per day for hand washing. Seventeen (19%) mothers reported hand washing within 1 hour before hand rinse sampling at the dry season visit, seven (8%) had visible dirt on the palms, and 52 (59%) had visible dirt underneath their nails.

The average number of times mothers reported washing their hands with soap the previous day and the number of mothers reporting washing their hands within the hour before having their hand rinse taken were significantly greater during the dry season versus the rainy season visit ($P \leq 0.05$). The average time reported by mothers since the last time they washed their hands with soap was significantly less at the dry versus the rainy season visit ($P < 0.01$). Mean FIB concentrations and turbidity of mothers' hand rinses, as well as the percentage of mothers with visible dirt on their palms or under their fingernails, were not significantly different at the dry season visit compared with the rainy season sampling ($P \geq 0.05$). The average liters per capita per day reportedly used for hand washing and the mothers' reported activity prior to hand rinse sampling were not significantly different by season ($P \geq 0.05$). Descriptive statistics and *P* values from bivariate analyses between each of the aforementioned hygiene behaviors and hand cleanliness characteristics during the rainy and dry seasons are presented in Table 2. Statistical analyses of NVGII results did not adjust for half of respondents receiving a hand hygiene behavior intervention between the rainy and dry season visits because the norovirus prevalence and hygiene parameters of interest did not change significantly between seasons in the hand hygiene intervention cohort compared with the control cohort (data not shown).

Reported health symptoms. At the rainy season sampling, four (5%) of respondents (mothers) reported having symptoms indicative of HCGI within the 2 days before sampling. Mothers also reported at the rainy season visit experiencing the following symptoms within the past 2 days: 14 (16%) reported having abdominal pain, 2 (2%) reported having three or more loose, watery stools within a 24-hour period, 0 (0%)

TABLE 2
Descriptive statistics of hygiene behaviors and characteristics of mothers' hands before sampling by season (N = 88 households)

Variable	Rainy	Dry	P†
Mean no. (SD) of times mother reported hand washing with soap the previous day*	3.0 (2.3)	3.7 (1.8)	0.03§
Mean (SD) LPCD used for hand washing*‡	2.0 (1.8)	1.8 (0.8)	0.48
No. (%) of respondents reported hand washing within 1 hour prior to hand rinse sampling*	22 (28.2)	45 (51.1)	< 0.01§
Mean time (hour) (SD) since respondent last wash their hands with soap*	2.8 (2.4)	1.7 (1.4)	< 0.01§
No. (%) of respondents reported activity before hand rinse sampling*			
Sitting	56 (63.6)	57 (64.8)	0.90
Washing (clothes, dishes, child)	12 (13.6)	9 (10.2)	
Hand washing	0 (0.0)	0 (0.0)	
Food preparation/eating/serving	12 (13.6)	14 (15.9)	
Other (gardening/farming, sweeping, using toilet, or helping child clean up after toilet)	8 (9.1)	8 (9.1)	
Mean $\log_{10}$ concentration (CFU/two hands) (SD) of fecal indicator bacteria on mother's hands			
Escherichia coli	2.2 (1.0)	2.1 (1.0)	0.33
Enterococcus spp.	2.6 (1.0)	2.6 (0.9)	0.82
Mean $\log_{10}$ turbidity (NTU) (SD) of mother's hand rinse	1.3 (0.5)	1.2 (0.3)	0.19
No. (%) of respondents/primary caregivers with dirt observed on their palms	12 (13.6)	7 (8.0)	0.23
No. (%) of respondents/primary caregivers with dirt observed under their fingernails	48 (54.6)	52 (59.1)	0.54

CFU = colony forming unit; LPCD = liters per capita per day; NTU = nephelometric turbidity unit; SD = standard deviation.
*Variable was self-reported by primary caregiver/mother.
†P values are the results of bivariate analyses between hygiene variables in the rainy and dry season. χ^2 or Fisher's exact tests were used to analyze associations and differences of proportions between binary variables. t tests were used to test differences of means between continuous variables.
‡Values Ln-transformed to perform t test.
§Significantly different at the 95% confidence level.

reported blood in their stool, 3 (3%) reported feeling nauseated, 2 (2%) reported vomiting, 14 (16%) reported having a fever, 16 (18%) reported having a constant cough, 18 (20%) reported having congestion, and 5 (6%) reported difficulty breathing. As dictated by the study household selection limitations, no children under the age of five years were reported to have symptoms of HCGI within the 2 days before the rainy season sampling. However, mothers reported seeing the following symptoms in the past 2 days for at least one of their children under the age of five years during the rainy season: 4 (5%) had abdominal pain, 0 (0%) had three or more loose, watery stools within a 24-hour period, 0 (0%) had blood in their stool, 0 (0%) felt nauseated, 0 (0%) vomited, 22 (25%) had a fever, 37 (42%) had a constant cough, 42 (48%) had congestion, and 6 (7%) had difficulty breathing.

At the dry season sampling, 4 (4%) households had one child under the age of five years with reported symptoms of HCGI within the previous 2 days. Though none of the mothers at the dry season visit reported symptoms of HCGI within the previous 2 days, mothers did, however, report having the following symptoms during the 2 days before the dry season sampling: 5 (6%) had abdominal pain, 0 (0%) had three or more loose, watery stools within a 24-hour period, 0 (0%) had blood in their stool, 0 (0%) were nauseated, 0 (0%) vomited, 6 (7%) had a fever, 4 (5%) had a constant cough, 10 (11%) had congestion, and 0 (0%) had difficulty breathing. Mothers also reported of seeing the following symptoms in the past 2 days in at least one of their children under the age of five years at the dry season visit: 0 (0%) had abdominal pain, 3 (3%) had three or more loose, watery stools within a 24-hour period, 0 (0%) had blood in their stool, 1 (1%) felt nauseated, 1 (1%) vomited, 14 (16%) had a fever, 26 (30%) had a constant cough, 43 (49%) had congestion, and 0 (0%) had difficulty breathing.

NVGII on hands of mothers. Human NVGII was detected in 4 (5%) mothers hand rinse samples collected during the rainy season, and the average concentration in those rinses with detectable norovirus was 1.8 (SD = 0.2) $\log_{10}$ NVGII copies per two hands (Table 3). NVGII was detected in 5 (6%) hand rinses sampled during the dry season, and of those rinses with detectable norovirus, the average concentration was 3.2 (SD = 0.9) $\log_{10}$ NVGII copies per two hands. The norovirus prevalence was not statistically different between the rainy and dry seasons (FET, P = 0.62). NVGII was not detected on the hands sampled during the dry season that had detectable amounts of NVGII during the rainy season, and vice-versa. Of the hand rinses with NVGII, the average $\log_{10}$-transformed concentration was significantly higher during the dry compared with the rainy season (t = 3.19, P = 0.03). None of the households with NVGII detected reported symptoms of HCGI in either the respondent or any children under the age of five years. NVGII was not detected in any of the three samples for which volumes of less than 100 mL were filtered.

Associations between NVGII, FIB, and turbidity of mothers' hand rinses. The concentrations of *Eschericia coli* (EC) and enterococci (ENT) were not significantly different when NVGII was detected versus not detected in all hand rinses analyzed (N = 176, both the rainy and dry season samples) (EC: mean difference = 0.1 log units, t = 0.2, P = 0.87; ENT: mean difference = 0.2 log units, t = 1.2, P = 0.25). The turbidities of hand rinses were also not significantly different when NVGII was detected versus not detected in all hand rinses analyzed (N = 176, mean difference = 0.2 log units, t = 1.2, P = 0.25).

Looking at the results by season, the concentrations of EC and ENT were not significantly different when NVGII was detected versus not detected in hand rinses collected during

TABLE 3
Concentration of NVGII on hands of mothers

Household no.	NVGII RNA copies per two hands	Season
1	124	Rainy
2	53	Rainy
3	60	Rainy
4	41	Rainy
5	13,397	Dry
6	154	Dry
7	18,884	Dry
8	656	Dry
9	371	Dry

NVGII = norovirus GII.
During rainy and dry seasons, NVGII was detected in nine unique households out of 88 total households (arbitrary household number provided).

the rainy season (EC: mean difference = 0.1 log units, t = 0.3, P = 0.78; ENT: mean difference = 0.8 log units, t = 3.8, P = 0.76) or the dry season (EC: mean difference = 0.0 log units, t = 0.1, P = 0.94; ENT: mean difference = 0.5 log units, t = 1.1, P = 0.29). The turbidity of hand rinses was also not significantly different when NVGII was detected versus not detected during the dry season (mean difference = 0.0 log units, t = 0.0, P = 0.98) but was significantly higher when NVGII was detected versus not detected during the rainy season (mean difference = 0.4 log units, t = 5.7, $P < 0.01$).

When only considering positive norovirus results, EC, ENT, and turbidity levels of all hand rinses with NVGII detected (N = 9) were not linearly correlated with NVGII concentrations (EC: $r_p = -0.06$, P = 0.88; ENT: $r_p = -0.00$, P = 1.00; turbidity: $r_p = -0.05$, P = 0.90). The concentration of NVGII on hands was not linearly correlated with FIB or turbidity concentrations during the rainy season (N = 4; EC: $r_p = -0.27$, P = 0.73; ENT: $r_p = -0.33$, P = 0.67; turbidity: r_p = 0.60, P = 0.40) or FIB during the dry season (N = 5; EC: r_p = −0.11, P = 0.86; ENT: $r_p = -0.40$, P = 0.54). Interestingly, turbidity of hand rinses with NVGII collected during the dry season was positively correlated with NVGII concentrations (N = 5, r_p = 0.88, P = 0.05).

All extraction blanks and NTCs were negative, and duplicate samples generally agreed for culture-based FIB analyses. The average ΔCt for the subset of hand rinse samples tested for inhibition was −0.14, and therefore, it was concluded that inhibition did not impact the RT-qPCR reactions.

DISCUSSION

The presence of NVGII detected in hand rinses collected during both the rainy and dry seasons in our study suggests that mothers' hands may serve as a source of norovirus exposure within the homes of young children in Tanzania. The prevalence of human NVGII on mothers' hands in Bagamoyo was similar to the previously reported prevalence of adenovirus, and approximately half the reported prevalence of rotavirus on mothers' hands living in the same area.[17,18]

The concentrations of NVGII on mothers' hands were found to be higher in hand rinses collected during the dry season (August–October) compared with those collected from mothers during the rainy season (March–May). The reason for this difference is unclear and is likely driven by high concentrations in two of the contaminated hand rinses collected during the dry season. A previous study in Bagamoyo found that reportedly higher volumes of water used for hand washing was associated with a significant decrease in the odds of detecting enteric viruses (either rotavirus, enterovirus, or adenovirus) on a mother's hands.[17] Therefore, one hypothesis for the possible seasonal concentration difference is that less water may be available to mothers for hand washing during the dry season. However, there was no significant difference in the amount of water reportedly used for hand washing between the seasons; in fact, reported hand washing behaviors were significantly improved in the dry relative to wet seasons. Thus, there may be other factors contributing to the concentration difference or the study design may be underpowered because of the low prevalence of norovirus on mother's hands in Tanzania. Future work should analyze a larger sample size of contaminated hand rinses to confirm and explain the seasonal concentration difference seen in this study.

FIB levels were not found to be associated with NVGII hand contamination. This is consistent with the previous work in Tanzania and Ghana that found culture-based FIB to be poor indicators of enteric virus presence (rotavirus, enterovirus, and/or adenovirus) on mothers' hands and NVGII presence in wastewater, respectively.[18,34] Turbidity was found to be associated with NVGII presence on mothers' hands, which is also consistent with the previous research that showed an association between turbidity and enteric virus presence in hand rinses in Tanzania.[18] However, in this study, the association between NVGII presence and hand rinse turbidity was only observed during the rainy season (when the hand rinses from the previous study that also saw an association between viral nucleic acid presence and hand rinse turbidity[18] were also collected). This suggests that turbidity may not serve as a consistent indicator for viruses on hands in tropical settings. Further research is needed to evaluate turbidity as an indicator for viral contamination on hands in low-income settings during various climate conditions.

There are several limitations to this study. First, the detection of viral RNA does not necessarily indicate the presence of infectious NVGII. However, given that human noroviruses are not currently cultivable,[35] research to determine infectious prevalence would need to be undertaken with a surrogate calicivirus[36] and, as such, may also not accurately reflect the prevalence of infectious norovirus on mothers' hands. Second, this study only represents a cross-sectional comparison of NVGII hand contamination during the rainy versus the dry season. Variation in hand contamination within each season is not captured with our study design. Moreover, results of this study are only representative of one site in Tanzania; additional work is needed to evaluate its relevance in other settings. Also, because of the sample size and low NVGII prevalence, the study may not have been powered to see a significant seasonal difference in NVGII prevalence on mothers' hands. Finally, the hand rinse method was used to detect viruses that are readily eluted in aqueous solutions (such as drinking water and saliva in a mouth). The recovery of viruses from hands via this hand rinse method has not been rigorously evaluated. Undertaking such a study would require seeding hands with viruses (or viral surrogates) and assessing recovery. Thus, although viral presence in hand rinses in this study suggests the presence of readily rinseable viruses (or viral RNA) on human hands, there could potentially be viruses remaining present on hands after the rinsing that could represent a potential additional health risk during food preparation or fomite handling.

Because of the limitations in sample availability, this study only selected households without reported symptoms of HCGI in children under the age of five years at the baseline visit (rainy season), which in turn may have biased the results. However, a recent cross-sectional study in the same area of Tanzania found no association between HCGI in children under the age of five years and viral nucleic acid presence (rotavirus, enterovirus, or adenovirus) on hands of mothers.[17] Future work should be done to verify the findings from this study to confirm that household selection bias did not occur.

This study only measured norovirus genogroup GII, whereas human norovirus infections can also be caused by genogroup GI and GIV.[2] Therefore, future research that

includes analysis of hand rinse samples for norovirus GI and GIV would be valuable. Also, this study was unable to find an association between traditional culture-based FIB and NVGII. A recent study in Tanzania found significant associations between the presence of an enteric virus (rotavirus, adenovirus, and/or enterovirus) and the presence of a molecular marker of human-specific *Bacteroidales* (BacHum[37]).[18] Furthermore, a study in Hawaii, United States, found a significant association between norovirus in environmental water samples and BacHum presence.[28] Therefore, future work should also include evaluating whether human-specific *Bacteroidales* may serve as a better indicator for norovirus presence on hands in Tanzania.

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