Rapid Active Sampling Surveys as a Tool to Evaluate Factors Associated with Acute Gastroenteritis and Norovirus Infection among Children in Rural Guatemala

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Abstract. We examined burden and factors associated with norovirus (NoV) acute gastroenteritis (AGE) among children in rural Guatemala. Children age 6 weeks to 17 years were enrolled into three AGE surveillance groups, using two-stage cluster sampling: a prospective participatory syndromic surveillance (PSS) cohort and two cross-sectional rapid active sampling (RAS) surveys, conducted from April 2015 to February 2016. Epidemiologic and NoV testing data were used to identify factors associated with NoV infection, AGE, and NoV+ AGE. The three cross-sectional surveys (PSS enrollment visit, RAS Survey 1, and RAS Survey 2) enrolled 1,239 children, who reported 134 (11%) AGE cases, with 20% of AGE and 11% of non-AGE samples positive for NoV. Adjusted analyses identified several modifiable factors associated with AGE and NoV infection. The cross-sectional RAS surveys were practical and cost-effective in identifying population-level risk factors for AGE and NoV, supporting their use as a tool to direct limited public health resources toward high-risk populations.

Noroviruses (NoVs) are a leading cause of acute gastroenteritis (AGE) worldwide, resulting in an estimated 685 million cases of diarrhea and 212,489 deaths annually.^{1,2} Low and middle income countries (LMICs), in which health care and public health infrastructure are limited, account for 85% of NoV-associated AGE and 99% of deaths.^{1,2} With no treatments available other than supportive care, preventive measures, including water and sanitation health and forthcoming vaccines, represent the most effective solutions in reducing the overall NoV disease burden.^{3–5} To identify priority groups for public health interventions against NoV, new simple and effective methods are needed to estimate disease burden in specific regions and to identify high-risk populations for both symptomatic and asymptomatic NoV infection.⁴

To better characterize the populations at greatest risk of NoV in a rural region of Guatemala, we analyzed epidemiologic data from three cross-sectional surveys performed over a 1-year period and identified risk factors associated with NoV infection and disease that could be used to guide public health interventions in the region.

This analysis was performed within a larger study⁶ comparing two surveillance methodologies to estimate NoV and AGE disease burden in resource-limited settings. The first system, a prospective participatory syndromic surveillance (PSS) cohort, obtained baseline data and stool samples from participating households, and then prospectively followed them for AGE using a smartphone-based symptom diary application (Integra IT, Bogota, Colombia). The second system used rapid active sampling (RAS) surveys, in which nurses performed two rapid (4–6 weeks) cross-sectional

surveys among two separate groups of households from the same catchment area at different time points in the year. Each study group (PSS cohort, RAS Survey 1, RAS Survey 2) was randomly selected using two-stage cluster sampling (30 clusters of seven households each).^{7,8} Costs for the surveys were minimized by randomizing in clusters, using a smartphone application for data collection, and performing all data and sample collection in the field followed by testing samples in a central laboratory. For comparability, we included the enrollment visit for the PSS cohort and the two RAS cross-sectional surveys in this analysis.

The study was conducted in 25 communities within a 200-km² catchment area along the coastal lowlands of south-west Guatemala. These communities have a high prevalence of food insecurity, poverty, low access to health care, and high levels of diarrheal and respiratory disease.⁹ Households with children 6 weeks to 17 years were eligible for study inclusion, and all eligible children within a household were offered enrollment during three attempted home visits. Households enrolled in the PSS cohort additionally had to demonstrate proficiency in using the smartphone application. Each household could only be enrolled into one of the three study groups.

Specimens were collected at the home using Copan FLOQSwabs™ (Brescia, Italy) either by rectal swab or fresh (< 2 hours old) stool sample and eluted in eNAT™ transport solution (Copan) before testing, with both collection techniques previously demonstrating similar molecular viral yield.^{10,11} Samples were placed into a –20°C freezer and transported frozen to Universidad del Valle de Guatemala (UVG) for diagnostic testing. For viral extraction, the specimens were gently mixed with 200 µL of the eNAT™ transport solution. Viral RNA was extracted using QIAamp® Viral RNA Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. A total of 60 µL of purified viral RNA was obtained and stored at –70°C until reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) analysis.

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Molecular testing for NoV was performed as previously described.¹² For each sample, single RT-qPCR reactions were performed for NoV genogroup I (GI) and genogroup II (GII) as well as for ribonucleoprotein as an internal control. The amplification of NoV GI was conducted using the primer set Cog 1F/Cog 1R and TaqMan[®] probe Ring 1C (Waltham, MA), whereas the detection of NoV GI was performed using primer set Cog 2F/Cog 1R and TaqMan[®] probe Ring 2 as previously described.¹³ A sample was positive if the cycle threshold values obtained were < 40.

Case definitions were created prior to study initiation. AGE was defined as self-reported vomiting or diarrhea for ≥ 3 days or both for ≥ 1 day in the preceding week, emphasizing the identification of more severe disease; NoV infection was defined as any child with a NoV PCR-positive stool or rectal swab; and NoV-associated AGE was defined as AGE with concurrent NoV infection at the time of sampling. Demographic and epidemiologic characteristics were compared using χ^2 tests for categorical variables, and analysis of variance for continuous variables. In separate models, we tested for the association between potential risk factors and three different outcomes (AGE versus no AGE, any NoV[+] versus NoV[-], and NoV[+] AGE versus NoV[-] AGE) using unadjusted and adjusted generalized linear models with a binary distribution and the log link function, unless model did not converge, in which case the logit function was used. Analysis of the PSS cohort used data from the enrollment visit only. SAS v 9.4 (Cary, NC) was used for all data analysis. The study was approved by Institutional Review Boards at the University of Colorado, the UVG, and the Guatemala Ministry of Health.

The PSS cohort enrolled 207 (47%) of 444 eligible households, including 469 children, from April to September

2015 (Table 1). The two RAS cross-sectional surveys were each completed more than 4–6 weeks in October to November 2015 and January to February 2016, with a total of 629 eligible households screened and 420 households (67%) enrolled, including 770 children. Eligible RAS households had a greater enrollment rate than PSS households (67% versus 47%, $P < 0.001$), and enrolled RAS households had more people per household (5.4 versus 5.0 people, $P = 0.006$), who were also younger ($P = 0.04$) and more often of nonindigenous ethnicity ($P = 0.003$). The most common reasons for declining participation ($N = 164$, 13%) included lack of perceived benefit to the child (42%), discomfort with rectal specimen collection (28%), and medical care obtained elsewhere (10%); 18% of households declined participation in the PSS study because they did not want responsibility for the smartphone.

Because of the cross-sectional nature of the data, potential risk factors were explored for association with AGE and NoV infection using prevalence ratios (PRs). Variables that were significantly associated with risk for AGE, NoV infection, and NoV+ AGE are summarized in Table 2. After adjusting for age, number of children ≤ 5 years in the household, and number of adults in the household, risk factors that were significantly associated with AGE included increased number of adults per household (PR = 1.1, $P = 0.02$), other children in the home with AGE (PR = 3.3, $P < 0.0001$), using piped water in the home (PR = 1.6, $P = 0.03$), or bottled water in the home (PR = 4.6, $P = 0.02$), not having a well (PR = 1.9, $P = 0.0004$), and having a younger age (PR = 1.1, $P < 0.0001$). Having other children in the home with AGE was significantly associated with NoV infection (PR = 1.8, $P = 0.048$), and breastfeeding was found to be protective against NoV infection (PR = 0.3,

TABLE 1
Characteristics of study participants in three cross-sectional surveys in the Coastal Lowlands of Southwest Guatemala, 2015–2016

Study population	PSS enroll	RAS Survey 1	RAS Survey 2	P value§
Dates	April to September 2015	October to November 2015	January to February 2016	
HH eligible, <i>n</i> (%)	444 (67)	351 (60)	278 (68)	0.01
HH enrolled, <i>n</i> (%)	207 (47)	210 (60)	210 (76)	< 0.001
Number of children enrolled, <i>n</i>	469	402	368	–
Number of children with stool sample, <i>n</i> (%)	265 (57)	211 (52)	203 (55)	0.30
Demographics				
Age, years (SD)*	7.3 (4.7)	7.3 (4.9)	6.5 (5.0)	0.04
Number of people in house, mean (SD)*	5.0 (1.8)	5.3 (2.1)	5.5 (2.2)	0.02
Number of < 8 years per HH, mean (SD)*	2.6 (1.4)	2.4 (1.5)	2.6 (1.4)	0.19
Number of ≤ 5 years per HH, mean (SD)*	1.0 (0.8)	1.0 (0.8)	1.0 (0.9)	0.99
Female, <i>n</i> (%)	225 (48)	210 (52)	188 (51)	0.41
Ladino, <i>n</i> (%)	452 (97)	383 (98)	367 (100)	0.003
Primary caregiver is literate, <i>n</i> (%)	183 (89)	176 (85)	183 (87)	0.59
Clinical data				
Number of stools per week, mean (SD)	13.4 (5.8)	13.6 (5.3)	13.1 (5.4)	0.39
Normal stool consistency				0.46
Formed/solid, <i>n</i> (%)	383 (82)	337 (84)	295 (80)	–
Soft, <i>n</i> (%)	82 (18)	60 (15)	71 (19)	–
Liquid, <i>n</i> (%)	4 (1)	5 (1)	2 (1)	–
AGE, <i>n</i> (%)‡	49 (10)	56 (14)	29 (8)	0.31
Sample available, <i>n</i> (%)	36 (73)	42 (75)	24 (83)	0.38
NoV+, <i>n</i> (%)†	4 (11)	6 (14)	5 (21)	0.31
Asymptomatic NoV, <i>n</i> (%)	25 (11)	20 (12)	15 (8)	0.36

AGE = acute gastroenteritis; HH = household; NoV = norovirus; PSS = participatory syndromic surveillance; RAS = rapid active sampling; SD = standard deviation. Comparison between children enrolled in three study groups: the PSS cohort (includes enrollment visit only), RAS Survey 1, and RAS Survey 2.

* Comparisons made assuming equal variance across the three groups.

† Mean and median cycle threshold value for NoV testing were 33.8 and 35.1, respectively.

‡ AGE defined as ≥ 3 days of vomiting or diarrhea, or one day of both; ≥ 1 day of vomiting or diarrhea would result in an additional 111 AGE cases would be included.

§ Demographic and epidemiologic characteristics were compared using χ^2 tests for categorical variables, and analysis of variance for continuous variables.

TABLE 2
Risk Factors for acute gastroenteritis among children living in the Coastal Lowlands of Southwest Guatemala, 2015–2016

Risk factors for AGE				
Risk factor	AGE (N = 134)	No AGE (N = 1,105)	Crude PR (CI)	Adjusted PR (CI)†
Age, year, mean (SD)	5.0 (4.1)	7.3 (4.9)	0.9 (0.9–0.9)*	0.9 (0.7–1.0)*
Number of children per HH ≤ 5 years, mean (SD)	1.4 (1.0)	1.1 (0.9)	1.30 (1.1–1.5)*	1.1 (0.9–1.3)
Number of adults per HH ≥ 18 years, mean (SD)	3.1 (1.5)	2.8 (1.3)	1.2 (1.0–1.3)*	1.1 (1.0–1.3)*
Other child in HH with AGE, n (%)	36 (27)	114 (10)	2.7 (1.9–3.3)*	3.3 (2.0–5.3)*‡
Breastfed (if age < 2 years), n (%)	41 (87)	161 (87)	0.8 (0.4–1.8)	0.6 (0.3–1.3)
Type of house				
Aluminum, n (%)	11 (8)	44 (4)	1.9 (1.1–3.4)*	1.7 (1.0–2.9)
Wood, n (%)	25 (19)	219 (20)	1.0 (0.7–1.5)	0.9 (0.6–1.4)
Other, n (%)	3 (2)	20 (2)	1.3 (0.4–3.7)	1.1 (0.4–3.3)
Cement block, n (%)	95 (71)	822 (74)	Ref	Ref
Water source				
Waterline, n (%)	34 (25)	204 (19)	1.5 (1.1–2.2)*	1.6 (1.0–2.6)*‡
Bottled water, n (%)	4 (3)	6 (1)	4.2 (1.9–9.2)*	4.6 (1.2–17.0)*‡
Other, n (%)	3 (2)	10 (1)	2.4 (0.9–6.5)	3.8 (1.0–14.8)*‡
Well, n (%)	93 (69)	885 (80)	Ref	Ref
Property exposures				
Well, n (%)	93 (69)	892 (81)	0.6 (0.2–0.8)*	0.5 (0.4–0.8)*
Septic tank, n (%)	78 (58)	754 (68)	0.7 (0.5–0.9)*	0.8 (0.6–1.1)
Natural water, n (%)	24 (18)	180 (16)	1.1 (0.8–1.7)	1.2 (0.7–1.9)
Risk Factors for NoV Infection				
Risk Factor	All NoV(+) (N = 75)	All NoV(–) (N = 588)	Crude PR (CI)*	Adjusted PR (CI)†
Age, y, mean (SD)	3.3 (3.2)	4.3 (3.4)	0.9 (0.8–1.0)*	1.7 (0.8–3.3)
Number of children per HH ≤ 5 years, mean (SD)	1.5 (0.7)	1.5 (0.9)	1.1 (0.8–1.4)	1.2 (0.7–1.9)
Number of adults per HH ≥ 18 years, mean (SD)	3.0 (1.6)	2.8 (1.3)	1.1 (1.0–1.3)	1.1 (0.8–1.3)
Other child in HH with AGE, n (%)	14 (19)	74 (13)	1.5 (0.9–2.6)	1.8 (1.0–3.3)*
Breastfed (if age < 2 years), n (%)	67 (89)	559 (95)	0.5 (0.2–0.9)*†	0.3 (0.1–0.9)*‡
Type of house				
Aluminum, n (%)	5 (7)	36 (6)	1.1 (0.5–2.6)	1.2 (0.5–2.8)
Wood, n (%)	17 (23)	129 (22)	1.0 (0.6–1.7)	1.1 (0.7–1.9)
Other, n (%)	1 (1)	14 (2)	0.6 (0.1–4.0)	0.6 (0.1–3.9)
Cement block, n (%)	52 (69)	410 (70)	Ref	Ref
Water source				
Waterline, n (%)	12 (16)	108 (18)	0.9 (0.5–1.6)	0.8 (0.5–1.5)
Bottled water, n (%)	2 (3)	4 (1)	2.9 (0.9–9.2)	2.4 (0.8–7.8)
Other, n (%)	0 (0)	7 (1)	NC	NC
Well, n (%)	61 (81)	469 (80)	Ref	Ref
Property exposures				
Well, n (%)	65 (81)	478 (80)	1.9 (0.9–3.8)	2.0 (1.0–4.3)
Septic tank, n (%)	52 (65)	402 (67)	0.9 (0.6–1.4)	1.0 (0.6–1.5)
Natural water, n (%)	18 (23)	100 (17)	1.3 (0.8–2.1)	1.3 (0.8–2.2)
Risk Factors for NoV+ AGE				
Risk factor	NoV(+) AGE (N = 15)	NoV(–) AGE (N = 87)	Crude PR (CI)	Adjusted PR (CI)†
Age, y, mean (SD)	2.2 (2.0)	4.0 (3.1)	1.0 (0.9–1.0)	0.7 (0.5–1.0)*‡
Number of children per HH ≤ 5 years, mean (SD)	1.6 (0.6)	1.6 (1.0)	0.3 (0.0–4.4)‡	0.8 (0.3–1.7)‡
Number of adults per HH ≥ 18 years, mean (SD)	3.6 (1.8)	3.0 (1.4)	0.9 (0.6–1.2)	1.4 (1.0–2.0)‡
Other child in HH with AGE, n (%)	4 (27)	20 (23)	0.9 (0.3–2.4)	3.5 (1.1–10.9)*
Breastfed (if age < 2 years), n (%)	10 (67)	73 (83)	0.5 (0.2–1.9)	0.7 (0.2–2.5)
Type of house				
Aluminum, n (%)	2 (13)	6 (7)	1.9 (0.5–7.3)	3.1 (0.9–11.4)
Wood, n (%)	4 (27)	19 (22)	1.3 (0.5–3.9)	2.0 (0.7–6.3)
Other, n (%)	0 (0)	3 (3)	NC	NC
Cement block, n (%)	9 (60)	59 (68)	Ref	Ref
Water source				
Waterline, n (%)	3 (20)	23 (26)	0.8 (0.2–2.7)	0.7 (0.2–3.0)‡
Bottled water, n (%)	2 (13)	1 (1)	4.7 (1.7–12.5)*	8.2 (0.6–104.1)‡
Other, n (%)	0 (0)	3 (3)	NC	NC
Well, n (%)	10 (67)	60 (61)	Ref	Ref
Property exposures				
Well, n (%)	11 (73)	63 (72)	1.1 (0.4–3.2)	1.1 (0.3–4.4)‡
Septic tank, n (%)	11 (73)	49 (56)	2.0 (0.7–5.9)	2.9 (0.8–11.2)‡
Natural water, n (%)	6 (40)	12 (13)	3.1 (1.3–7.6)*	6.2 (1.6–24.3)*‡

AGE = acute gastroenteritis; CI = confidence interval; HH = household; NC = not calculable; NoV = norovirus; OR = odds ratio; PR = prevalence ratio; SD = standard deviation. Selected epidemiologic risk factors associated with AGE, NoV infection (symptomatic or asymptomatic), and NoV-associated AGE in children 6 weeks to 17 years of age in rural Guatemala.

* P value < 0.05.

† Adjusted for age, number of adults, and number of children ≤ 5 years old in the household.

‡ Used the Logit function to estimate OR, since the PR was not estimable in the adjusted model.

$P = 0.02$) for children under 2 years. Having other children in the home with AGE ($PR = 3.53$, $P = 0.03$) and having natural water on one's property ($PR = 6.2$, $P = 0.009$), were significant risk factors for NoV+ AGE.

Cross-sectional RAS surveys were shown to be a practical tool to quickly ascertain AGE and NoV burden of disease and to identify groups at increased risk of infection and disease. The RAS surveys used to collect the burden of disease and risk factor data were timely and required limited resources. Each RAS survey was able to enroll 210 households in 4–6 weeks with only seven study nurses. Samples were collected in the field, processed in a local laboratory, and then tested at a reference laboratory at the end of the survey period, which minimized the technical expertise required at the surveillance site. The two-stage cluster randomization scheme allowed for efficient enrollment while still maintaining randomization and the collection of representative population-level data.

In this region of Guatemala, the RAS surveys demonstrated that using an individual household well for water collection was protective against AGE, whereas piped water, typically from larger community wells, was a risk factor for AGE. In an area with many outdoor latrines and frequent flooding, having natural water on one's property was associated with increased risk for NoV(+) AGE. Septic tanks were protective in the unadjusted analysis. These observations are supported by a previous survey in the same communities in which fecal contamination of well water was associated with recent rainfall and a larger well catchment area.¹⁴ Several other risk factors were also identified for AGE and NoV infection, including household crowding, younger age, aluminum housing (associated with poverty), and lack of breastfeeding. While several of these factors have previously been identified as AGE and NoV risk factors in other studies,² being able to provide local data may be useful in tailoring public health messaging to a community. The risk of AGE associated with bottled water is under investigation.

The study had several limitations. Our strict case definition for AGE may have biased our analysis to more severe disease that may carry a larger public health priority. Cross-sectional surveys collecting self-reported retrospective clinical data may be subject to reporting bias.¹⁵ However, we only asked about AGE symptoms in the preceding week (thus the recall period was quite short) and our prevalence was similar to those reported in Guatemala¹⁶ and other LMICs.^{17–21} Many subjects declined to provide a stool sample for NoV testing, which limited our power to identify risk factors for NoV infection and NoV+ AGE.

In conclusion, RAS surveys were effective and practical for characterizing AGE and NoV burden of disease and identifying high-risk groups within a community that could be targeted with limited public health resources. Pathogen-specific AGE burden of disease studies can take advantage of RAS surveys as a simple tool to identify disease-associated risk factors, and to measure the impact of public health interventions at a population level.

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