Am. J. Trop. Med. Hyg., 97(3), 2017, pp. 937–943
doi:10.4269/ajtmh.16-0799

Risk Factors for Norovirus Gastroenteritis among Nicaraguan Children

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Abstract. Norovirus is a leading cause of pediatric gastroenteritis. Understanding norovirus epidemiology is essential for reducing disease burden. We conducted a case–control study to describe the distribution, clinical features, and risk factors of norovirus gastroenteritis among children < 5 years of age in León, Nicaragua. Cases were children testing positive for norovirus and controls were children living in the cases' communities. Study staff interviewed mothers of enrolled cases and controls to obtain detailed exposure information including food, water, and sanitation sources; recent exposures; household characteristics; and handwashing practices. In addition, study staff requested stool samples to be tested for norovirus from select household members. We used descriptive statistics to understand the epidemiologic and clinical features of gastroenteritis episodes. To analyze potential risk factors, we used Firth's penalized logistic regression to estimate crude and adjusted odds ratios (ORs) and corresponding 95% confidence intervals (CIs). There were 102 children with gastroenteritis, 18 cases of norovirus and 31 controls. Norovirus cases occurred later in the year, corresponding to a delay in the rainy season. Cases were more likely to have a household member with norovirus in their stool as compared with controls [crude OR: 13.3 (95% CI: 2.5, 136.2) and adjusted OR: 11.5 (95% CI: 1.6, 223.2)]. In addition, alcohol-based hand sanitizer use among household members was reported for 10 (32%) of controls and but never for cases. Further research is needed to understand household transmission of norovirus in low- and middle-income countries and the potential impact of hand sanitizer use.

INTRODUCTION

Acute gastroenteritis is an important cause of morbidity and mortality among children < 5 years of age in low- and middle-income countries (LMICs). Prior to rotavirus vaccine introduction, rotavirus was the leading cause of severe gastroenteritis in children in Nicaragua with approximately 30% of all children hospitalized with gastroenteritis having rotavirus in their stool.[1] Because of the large number of children experiencing severe rotavirus gastroenteritis, the rates of severe gastroenteritis were expected to decline dramatically after the introduction of rotavirus vaccines in 2006.[2] However, despite the nearly 90% coverage of rotavirus vaccines,[3] rates of acute gastroenteritis remain high in Nicaragua.[4–6]

Norovirus is now the leading cause of medically attended pediatric viral gastroenteritis.[5] Although norovirus is often considered a mild gastrointestinal disease, it was detected among 26% of Nicaraguan children with severe dehydration during an episode of gastroenteritis[5] and accounts for 12% of severe episodes of gastroenteritis in children < 5 years and 200,000 child deaths in LMICs annually.[7] In fact, the prevalence of norovirus among Nicaraguan children hospitalized for gastroenteritis has increased from 15% to 24% in the pre- to postrotavirus vaccine era.[5,8]

Understanding the epidemiology and transmission dynamics of norovirus is essential to enhance public health efforts for making informed decisions to reduce the spread of disease. Norovirus is known to be highly contagious[9] and can be spread via person-to-person contact and contaminated food, water, and surfaces.[9,10] Symptoms of infection typically last 2–3 days[10]; however, viral shedding can occur 3–14 hours before symptom onset[11] and can persist for 3–4 weeks, or even longer, after symptoms resolve.[12] Both symptomatic and asymptomatic infected people can shed a high concentration of viral particles, > 10^6 particles per gram of feces.[8,13] Additionally, norovirus immunity is thought to be temporary and possibly genotype specific, which means that persons of any age may become infected and transmit the disease despite having had a previous norovirus infection.[10]

Although there have been some studies of risk factors of norovirus gastroenteritis conducted in high-income countries (HICs), little is known about norovirus risk factors in LMICs. Studies conducted in HICs have found that cases were more likely to have direct contact with persons with gastroenteritis than controls.[14–16] This direct person-to-person transmission was reported in 54% of the cases in children < 5 years of age in England.[14] These contacts occurred both inside and outside of the home but were more likely to be contacts outside the home.[14–16] Both familial contacts as well as outside contacts were important in a Swiss study, which reported the presence of family "mini outbreaks" with a high proportion of cases reporting contact with ill family members.[16] Given that person-to-person transmission is an important risk factor of norovirus in both England and Switzerland, it is possible that person-to-person contact within the household is an important route of transmission for norovirus in Nicaragua. However, due to differences in social and environmental conditions in Nicaragua, it is possible other environmental exposures are more important.

We conducted a case–control study to better characterize norovirus gastroenteritis among children in León, Nicaragua's second largest city with a population of about 200,000 inhabitants.[17] Most households in León have access to indoor

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municipal piped water, an indoor toilet, and a cement or brick floor.[18] Nicaragua was the first LMIC to introduce the rotavirus vaccine in 2006. Coverage of the vaccine is high with more than 90% of children receiving at least one dose of the vaccine.[3] This country is tropical and typically has annual peaks of norovirus infections during the beginning of the rainy season (May–August).[5,8] Our objective was to investigate the distribution, clinical features, and risk factors for medically attended norovirus gastroenteritis among children < 5 years of age living in León. Specifically, we sought to determine if there was an association between norovirus-infected children and norovirus-infected household members to understand if person-to-person contact within the household is a critical point of norovirus transmission in this setting.

MATERIALS AND METHODS

We conducted a case–control study from June to December 2015. Cases were defined as children < 5 years of age with medically attended norovirus gastroenteritis. Controls were children < 5 years of age living in the same neighborhood as the case and were free of norovirus gastroenteritis. Controls were allowed to have asymptomatic norovirus infection or gastroenteritis not due to norovirus. One to three community controls were recruited for each norovirus gastroenteritis confirmed case. Gastroenteritis cases were recruited from the emergency room of the public referral hospital in León (Hospital Escuela Oscar Danilo Rosales), a health center (Perla Maria Centro de Salud), and two of the health center's associated satellite health posts (Ruben Dario and Antenor Sandino). Any child < 5 years of age presenting with gastroenteritis, defined as forceful vomiting and/or ≥ 3 stools that were looser than normal during a 24-hour period, was recruited for the study and screened for norovirus. During a pilot enrollment period in May and June, only children < 2 years of age were screened; due to the low number of overall gastroenteritis cases, this age range was expanded to include any child < 5 years for the remainder of the study.

Any child < 5 years of age who sought medical treatment of gastroenteritis at one of the previously mentioned health facilities was recruited for the study. A stool sample was collected from the sick child along with a short questionnaire related to clinical symptoms. If the stool sample was positive for norovirus, study staff visited the household of the norovirus gastroenteritis case within 1–3 days to obtain more detailed exposure information including food consumption, water sources, sanitation sources, recent exposures, household characteristics, and handwashing practices. The mother of the case completed an interview questionnaire with a study staff member. In addition, the study staff member observed conditions within the household and requested stool samples from the following household members: children < 10 years of age living in the household, the mother, the father, the primary caregiver of the child, the primary food preparer, and any household member reported as having gastroenteritis in the past 14 days. Stool samples were collected from the household members in the following 2–3 days and were transported to the University of Nicaragua, León (UNAN-León) microbiology laboratory where they were processed, stored, and later tested for norovirus. After the case information was collected, study staff recruited one to three children from the neighborhood of the case to serve as controls. Mothers of controls completed the same interview questionnaire with a staff member. Similarly, study staff observed control household conditions and requested stool samples from the same household members listed earlier. All study participants, or their legal guardians, provided written informed consent. The study was approved by the Institutional Review Boards of the UNAN-León (Acta no. 19, 2015) and the University of North Carolina at Chapel Hill (Study no: 14-2149).

Stool samples were collected in sterile plastic containers or transferred to sterile plastic containers from soiled diapers. Samples were transported at 4°C to the microbiology laboratory within 24 hours of collection. A 10% (w/v) suspension of sample with phosphate-buffered saline (pH = 7.2) was prepared and stored at −20°C for reverse transcription polymerase chain reaction (RT-PCR) and nucleotide sequence analysis. To obtain testing results from cases within 24 hours, stool samples were tested for norovirus the same day of sample collection using a commercial rapid test according to the manufacturer's instructions (RIDA®QUICK (N1402) Norovirus, R-Biopharm AG, Darmstadt, Germany). Those testing positive for norovirus were considered cases and those testing negative were excluded from further study activities.

Norovirus status for controls and household members was determined by real-time RT-PCR for either genogroup I or II. Viral RNA was extracted from 200 μL of 1:10 stool suspensions using High Pure Viral RNA Kit (Roche Diagnostic GmbH, Mannheim, Germany) following the manufacturer's instructions. Real-time RT-PCR was performed using GoTaq® 1-Step RT-qPCR System (Promega, Madison, WI). In brief, 4 μL of RNA was added to a reaction mixture consisting of 10 μL of GoTaq qPCR Master Mix 2×, 0.4 pmol of either GII (NVG2 and COG2R) or GI (NVG1 and NVG1R) primers,[19,20] 0.4 μL of GoScript™ RT Mix, 1.6 μL of $MgCl_2$ (25 mM), 0.3 μL of CXR Reference Dye, and 2.7 μL of RNAse free water, to a final volume of 20 μL. The real-time PCR reactions were performed in a 96-well reaction plate using the Light Cycler® 96 Real-Time PCR System (Roche Diagnostic GmbH, Mannheim, Germany). PCR was performed under the following conditions: 37°C for 15 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 10 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. Melting curve analysis, to confirm amplicon specificity, was performed immediately after PCR completion. A sample was considered norovirus positive for GI and/or GII if the Ct value was ≤ 40 and the Tm was 80 ± 1°C for GI and 83 ± 1°C for GII. Nucleotide sequencing of the N-terminal and shell region of the capsid gene was performed by Macrogen Europe (Amsterdam, Netherlands). The sequencing reaction was based on BigDye chemistry (Macrogen, Amsterdam, The Netherlands); NVG2f2 forward primers and G2SKR reverse primers were used as sequencing primers for norovirus GII.[20,21] Genotypes were determined by submitting the sequences to the Norovirus Genotyping Tool, Version 1.0.[22]

We used descriptive statistics to understand the epidemiologic and clinical features of gastroenteritis episodes. We assessed the frequency of gastroenteritis and norovirus gastroenteritis episodes over the course of the study. We also described and compared the frequency of clinical symptoms among those with norovirus and nonnorovirus gastroenteritis using a Fisher's exact test. The distribution of norovirus

genotypes was tabulated among those children with norovirus gastroenteritis.

We were concerned about misreporting of risk factors related to self-reported handwashing behaviors. Therefore, prior to analyzing any risk factors, we assessed the validity of self-reported handwashing behaviors by determining the association between responses to negative control questions (e.g., questions about handwashing when we expect the answers to typically be that the person does not wash their hands, such as, do you wash your hands before going outside?) and answers to traditional handwashing questions (e.g., questions about handwashing when we expect the answer to be that the person does wash their hands, such as, do you wash your hands after using the toilet?). Associations were assessed using Fisher's exact test. Any responses to handwashing questions that were associated with responses to any negative control questions ($\alpha = 0.05$) were excluded from the risk factor analysis.

Finally, we used descriptive statistics to compare the demographic characteristics of cases and controls and then determined risk factors of norovirus gastroenteritis. To analyze risk factors, we used Firth's penalized logistic regression to estimate crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for risk factors. Firth's penalized logistic regression can partially correct for bias due to small sample size by using a penalized maximum likelihood for parameter estimation. This method produces less inflated and more stable estimates of ORs than traditional logistic regression and is therefore more conservative. We analyzed 18 risk factors that were chosen a priori and related to recent exposures, household characteristics, and hand hygiene practices. Those with a statistically significant OR ($\alpha = 0.05$) after adjustment for age and gender were included together in the multivariable model to estimate adjusted ORs. Complete case analyses were performed. All analyses were performed using SAS, version 9.4 (SAS Institute, Cary, NC). The main risk factor of interest was presence of ≥ 1 household member with norovirus in their stool.

RESULTS

Over the course of the study, there were 102 episodes of gastroenteritis among children < 5 years of age among those visiting the previously mentioned medical facilities in León. There was a bimodal distribution of gastroenteritis cases with peaks in mid-August and mid-October (Figure 1). Across all episodes of gastroenteritis, diarrhea was the most common symptom (90%) followed by fever (44%) and vomiting (30%).

There were 18 episodes of norovirus gastroenteritis among the 102 participants with gastroenteritis (18%). As shown in Figure 1, most of the norovirus gastroenteritis episodes occurred later in the 6-month study (October–November). Of the 18 norovirus gastroenteritis cases, 100% experienced diarrhea, about 50% vomiting, and about 30% fever (Table 1). Vomiting occurred more frequently among those with norovirus gastroenteritis as compared with those with non-norovirus gastroenteritis. All norovirus cases were infected with viruses belonging to genogroup II, with exception of one coinfection with GI:GII. The norovirus genotype was identified in 12 of the 18 norovirus cases. Of these, 10 were GII.4 and 2 were GII.2. All 10 GII.4 were assigned to the Sydney variant according to nucleotide sequence analysis.

There were 18 norovirus cases and 31 controls included in the final analyses. Cases and controls were similar in their gender distribution, almost all had their mother as their primary caregiver, and all lived in a household with electricity. Controls were slightly older and with more variability in their ages as compared with cases. In addition, a total of 42 household members of cases (median = 2; range = 1–5) and 50 household members of controls (median = 2; range = 0–4) were enrolled and provided stool samples for analysis. Of these 92 household members, eight had norovirus in their stool. Of these eight, none reported having symptoms of diarrhea or vomiting in the last 14 days; however, this information appears to have been subject to misreporting. No more than one household member, not including the index case, per household was positive for norovirus.

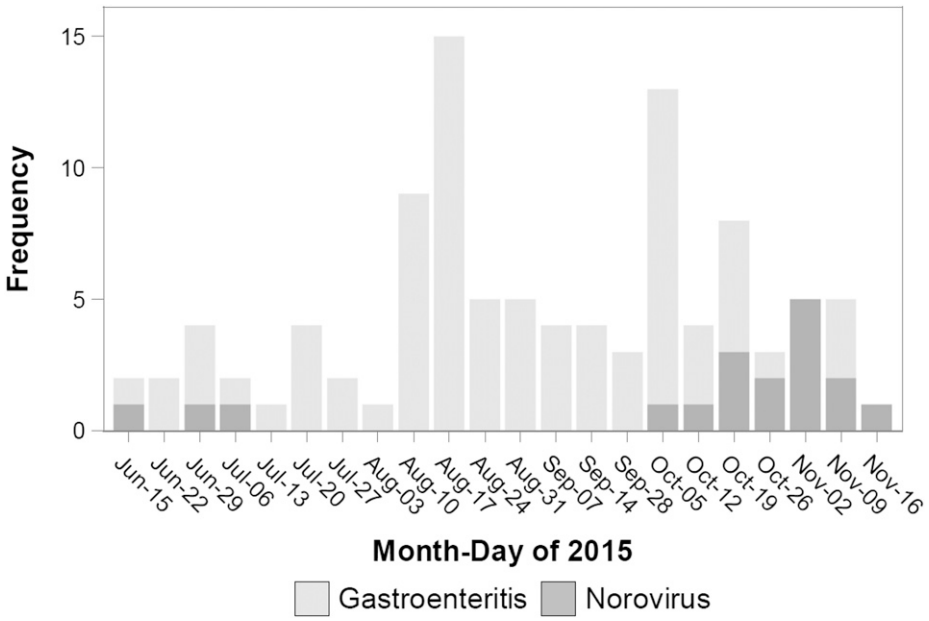

FIGURE 1. Distribution of gastroenteritis episodes over the 6-month study period.

TABLE 1
Clinical characteristics of children with norovirus and nonnorovirus gastroenteritis

	Norovirus (N = 18)	Nonnorovirus (N = 84)	
	N (%)	N (%)	Fisher's exact P value
Vomiting			0.02
Yes	10 (56)	20 (24)	
No	8 (44)	62 (76)	
Diarrhea			0.20
Yes	18 (100)	71 (88)	
No	0	10 (12)	
Bloody diarrhea			0.66
Yes	2 (11)	7 (8)	
No	16 (89)	77 (92)	
Fever			0.28
Yes	5 (29)	38 (47)	
No	12 (71)	43 (53)	
Oral rehydration			0.07
Yes	14 (78)	43 (52)	
No	4 (22)	40 (48)	
IV rehydration			0.55
Yes	1 (6)	3 (4)	
No	17 (94)	79 (96)	

IV = intravenous

Prior to the risk factor analysis, we assessed the validity of handwashing data. During interviews, all mothers reported members of their household always wash their hands after using the bathroom and that household members usually use soap when they wash their hands. However, when negative control questions were asked in conjunction with standard handwashing questions, there was more variation in responses given about handwashing practices. Nonetheless, most mothers interviewed still replied they wash their hands often after most tasks, even times we did not expect them to wash their hands (Supplemental Table 1). Because of suspected mismeasurement, we excluded many handwashing variables from the risk factor analysis.

After adjustment for age and gender, presence of a household member with norovirus in their stool was statistically significantly associated with case status (Table 2). In addition, 10 (32%) of the control mothers reported that household members use alcohol-based hand sanitizer, whereas no case mothers reported use among household members. Both household density (> 1 versus ≤ 1 persons per room) and eating outside the household in the past 14 days were marginally statistically significantly related to case status, P value = 0.11 and P value = 0.07, respectively.

DISCUSSION

This was a case–control study to describe the distribution, clinical features, and risk factors of norovirus gastroenteritis among children < 5 years of age in León, Nicaragua. In our study, 18% of children < 5 years of age seeking medical attention for gastroenteritis were infected with norovirus. We found that children with symptomatic norovirus infections (cases) were more likely to have a household member with norovirus in their stool than uninfected community-matched controls. In addition, alcohol-based hand sanitizer use among household members was reported to be much lower among cases as compared with controls.

The overall incidence of gastroenteritis was somewhat lower than expected for the 6-month study period. A study conducted in León during the same time frame (June–December) in 2005 reported almost four times the number of gastroenteritis episodes.[8] Similarly, a more recent case study from 2010 reported about one and half times the number of gastroenteritis episodes in that time frame.[5] However, the overall incidence of norovirus was comparable to previous studies that reported 11–24% norovirus incidence among children with gastroenteritis.[5,8,18] The timing of these norovirus gastroenteritis cases was delayed in our study as compared with prior studies that reported peak norovirus cases occurring May–July, coinciding with the start of the rainy season.[5,8] Interestingly, the rainy season was significantly delayed in Nicaragua in 2015, attributed to the El Niño-Southern Oscillation cycle. The delay in the rainy season may have been, at least in part, responsible for the low incidence of all-cause and norovirus gastroenteritis incidence during the initial months of the study. Both all-cause and norovirus gastroenteritis incidence increased following the eventual start of the rainy season. Heavy rainfall, either directly (e.g., through direct contact with contaminated water/flooding) or indirectly (e.g., changes in behaviors because of heavy rainfall), may be particularly important for norovirus transmission in this area.

The genotypic distribution and clinical manifestations of norovirus gastroenteritis cases were as expected based on previous studies. We identified GII.4 as the most prevalent norovirus genotype. This was similar to a previous community and hospital-based case study in Nicaragua, where GII.4 was found to be the most prevalent genotype of norovirus observed in young children with gastroenteritis.[8] GII.4 is also known to be the most common genotype currently circulating in Latin America[23] and the world.[24,25] Similarly, symptoms of infection were similar to those described in the literature. Diarrhea and vomiting were the most frequent symptoms reported among those with norovirus. In addition, vomiting was more common among those with norovirus gastroenteritis than with nonnorovirus gastroenteritis. This is consistent with the study, also from Nicaragua, by Becker-Dreps and others,[18] which reported vomiting was more common among those infected with caliciviruses (i.e., norovirus or sapovirus) compared with those not infected with a calicivirus. Interestingly, there were two cases that reported the presence of bloody diarrhea. Since norovirus is not known to cause bloody diarrhea, these children were likely coinfected with another enteric pathogen.

Cases were more likely to have a household member with norovirus in their stool than were controls. This demonstrates, in a research setting in a LMIC, multiple norovirus infections are commonly found in the same household. However, this does not imply household members infected cases or vice versa. Cases in the same household could be unrelated or occur from exposure to a common source (e.g., contaminated food, water, or surfaces). This observed association is consistent with studies in other settings where transmission of norovirus among household members has been documented including Ecuador,[26] Switzerland,[16] and the United Kingdom.[27] Although household transmission has been documented, the exact mechanism through which this occurs (i.e., direct exposure/contact to the sick individual or indirect exposure through a commonly contaminated food/surface) has not been elucidated. In addition, most studies have identified norovirus cases through active surveillance of norovirus

TABLE 2
Crude and adjusted odds ratios corresponding to the associations between norovirus gastroenteritis and specific risk factors

Potential risk factor	Case (*N* = 18) *N* (%)	Control (*N* = 31) *N* (%)	Crude OR (95% CI)	Adjusted* OR (95% CI)
Demographic characteristics				
Age (months)				
< 6	2 (11)	6 (19)	Referent	
≥ 6 to < 12	10 (56)	6 (19)	4.20 (0.79, 29.03)	
≥ 12 to < 18	1 (6)	13 (42)	0.29 (0.02, 2.62)	
≥ 18 to < 60	5 (28)	6 (19)	2.20 (0.36, 16.23)	
Gender				
Female	8 (44)	13 (42)	Referent	
Male	10 (56)	18 (58)	0.90 (0.29, 2.87)	
Household members				
Household members with norovirus in stool				
No	11 (61)	30 (97)	Referent	Referent
Yes	7 (39)	1 (3)	13.26 (2.50, 136.21)	11.46 (1.59, 223.23)†
Density (persons per room)				
≤ 1	5 (29)	14 (48)	Referent	Referent
> 1	12 (71)	15 (52)	2.13 (0.64, 7.74)	3.57 (0.84, 18.95)
Household members use alcohol-based hand sanitizer				
No	18 (100)	21 (68)		
Yes	0	10 (32)		
Mother washes hands before preparing food				
No	5 (28)	6 (19)	Referent	Referent
Yes	13 (72)	25 (81)	0.63 (0.17, 2.40)	0.89 (0.20, 3.96)
Mother washes hands after using the toilet				
No	3 (17)	4 (13)	Referent	Referent
Yes	15 (83)	27 (87)	0.72 (0.16, 3.62)	1.23 (0.18, 8.92)
Case/control				
Shared bottle				
No	4 (24)	9 (30)	Referent	Referent
Yes	13 (76)	21 (70)	1.33 (0.37, 5.31)	4.60 (0.58, 46.20)
In the past 14 days				
Attended social gathering				
No	5 (28)	11 (37)	Referent	Referent
Yes	13 (72)	19 (63)	1.45 (0.43, 5.23)	3.14 (0.61, 19.95)
Ate food outside home				
No	8 (44)	20 (65)	Referent	Referent
Yes	10 (56)	11 (35)	2.20 (0.70, 7.20)	4.84 (1.07, 30.27)
Used public transportation				
No	3 (17)	7 (23)	Referent	Referent
Yes	15 (83)	24 (77)	1.36 (0.34, 6.29)	3.04 (0.61, 20.74)
Household				
Drinking water source				
Home municipal water	14 (78)	26 (84)	Referent	Referent
Other	4 (22)	5 (16)	1.50 (0.35, 6.14)	0.91 (0.19, 4.08)
Toilet facility				
Indoor bathroom	13 (72)	22 (71)	Referent	Referent
Latrine	5 (28)	9 (29)	0.97 (0.26, 3.32)	1.02 (0.24, 4.38)
Floor material				
Other	15 (83)	29 (94)	Referent	Referent
Brick	3 (17)	2 (6)	2.67 (0.47, 17.54)	2.81 (0.34, 24.46)
Wall material				
Brick/cement	13 (76)	26 (84)	Referent	Referent
Cardboard/plastic/metal	4 (24)	5 (16)	1.61 (0.37, 6.65)	1.53 (0.33, 7.38)
Any animals inside				
No	5 (28)	10 (33)	Referent	Referent
Yes	13 (72)	20 (67)	1.26 (0.37, 4.57)	1.21 (0.27, 5.53)
Dogs inside				
No	7 (39)	16 (53)	Referent	Referent
Yes	11 (61)	14 (47)	1.74 (0.55, 5.74)	1.20 (0.30, 4.64)
Cats inside				
No	13 (72)	23 (77)	Referent	Referent
Yes	5 (28)	7 (23)	1.28 (0.34, 4.63)	0.88 (0.19, 4.03)
Chickens inside				
No	14 (78)	23 (77)	Referent	Referent
Yes	4 (22)	7 (23)	0.97 (0.24, 3.65)	0.51 (0.09, 2.56)
Pigs inside				
No	15 (83)	25 (83)	Referent	Referent
Yes	3 (17)	5 (17)	1.05 (0.22, 4.54)	0.51 (0.09, 2.73)

CI = confidence interval; OR = odds ratio.
* Adjusted for age and gender.
† Adjusted for age, gender, and total number of household members tested for norovirus.

gastroenteritis in a health center, which precludes researchers from understanding the household groups (e.g., school-aged children) that are the primary introducers of norovirus into households under typical conditions. More research is needed to understand how norovirus may spread within households and what interventions can best prevent the spread of disease.

Self-reported use of alcohol-based hand sanitizer by household members occurred among household members of some controls but never among household members of cases. Because of complete separation of these groups, we were unable to estimate an OR for this effect. Alcohol-based hand sanitizer use may be associated with other behaviors (e.g., thorough and frequent handwashing) that protect against norovirus infection. Alternatively, alcohol-based hand sanitizers may reduce the transmissibility of norovirus. In a few studies, alcohol-based hand sanitizers have been shown to reduce the viral levels of human norovirus surrogates (i.e., murine and feline caliciviruses).[28,29] However, the low infectious inoculum of norovirus[9] would limit attempts to determine if a reduction in viral load would translate to reduced transmissibility. Also, the precise similarity between human norovirus surrogates and true human noroviruses is unknown, because human noroviruses cannot be cultured.[30] In addition, some researchers claim soap and water provide better protection against norovirus than alcohol-based hand sanitizers,[31] and one paper even found the use of alcohol-based hand sanitizer was associated with increased risk of norovirus outbreaks in long-term care facilities in the United States.[32] The potential role of hand sanitizer needs to be explored in greater depth.

In addition, household density (> 1 versus ≤ 1 persons per room) and eating outside the household in the past 14 days were associated with norovirus case status. Crowding has been found to be a risk factor of gastroenteritis in a U.S. daycare setting.[33] A higher density of household members could result in more contact among household members and shared surfaces. In addition, this density measure was largely driven by the number of household members (> 1 versus ≤ 1 persons per room correlated very strongly with household sizes ≥ 4 versus 2–3 household members in a household). It is possible the exposure to more household members, regardless of density, is the driving factor. This merits further investigation. Furthermore, recent exposure to food outside of the home was associated with norovirus case status. This result is interesting to note, because contamination by food handlers is an important cause of norovirus infections and outbreaks in HICs[34,35] and may also be important in Nicaragua.

There are some limitations to this research study. The limited sample size resulted in imprecise estimates for the ORs. Future studies, with larger sample sizes, should explore these risk factors more fully with more rigorous control for potential confounding, which could not be fully accounted for in this study. Also, due to logistical constraints, the norovirus rapid test RIDA QUICK was used to identify norovirus gastroenteritis cases in real time so that exposure information and household samples could be collected soon after case identification. The sensitivity of this test is 87%.[36] This means some children with gastroenteritis who were screened could have had norovirus, but may have not tested positive and were not included in the case–control study. However, the specificity of the test is very high, 97%[36]; therefore, most likely all norovirus cases were true cases. In addition, we were unable to test stool samples for other common enteric pathogens. As a result, some norovirus gastroenteritis cases may have symptoms due in part or fully to other enteric pathogens. If these children had a norovirus infection several days earlier, it is possible their exposures have changed, and the effect of important exposures may be diluted by including these children among norovirus gastroenteritis cases in which no other enteric pathogen is detected. Furthermore, this study relied on many self-reported exposures, such as handwashing and hand sanitizer use, which could be subject to recall or social desirability bias. Ideally, a cohort study would be performed such that all exposure data, including detailed questionnaires, environmental samples, and biological specimens, would be collected prior to onset of gastrointestinal symptoms in the child. Similarly, samples from all household members would be collected prospectively to better understand the transmission dynamics of norovirus within households.

This study also has many strengths. To our knowledge, this is the first study in a LMIC to analyze risk factors of norovirus gastroenteritis among children. In addition, this study collected important exposure information, including stool samples from household members, in near real-time, rather than relying on self-reported exposure. Moreover, this study provides important information on the distribution of exposures among children with and without norovirus and can provide hypotheses for further research in this area. Though limited by small sample size, we showed that exposure to a norovirus-infected household member is associated with symptomatic norovirus infection in young children and alcohol-based hand sanitizer may have the potential to decrease children's risk of norovirus gastroenteritis.

In this case–control study of norovirus gastroenteritis, cases were more likely to have a household member with norovirus in their stool as compared with controls. In addition, alcohol-based hand sanitizer use among household members was not reported among cases but was reported among a third of controls. Further research is necessary to understand the occurrence and transmission of norovirus in households and the potential impact of hand sanitizer use.

Received October 11, 2016. Accepted for publication May 7, 2017.

Published online July 10, 2017.

Note: Supplemental information and table appear at www.ajtmh.org.

Acknowledgments: We would like to thank the study participants for their time and willingness to participate. We would also like to express gratitude to Jhosseling Delgado, Jayrintzina Palacios, Eveling Martinez for their assistance with data collection and to Johan Nordgren for his work in collaboration with the UNAN laboratory for the sequencing analysis.

Financial support: This research was funded by the University of North Carolina Institute for Global Health and Infectious Diseases.

Disclosures: Sylvia Becker-Dreps is receiving an investigator-initiated research award from Pfizer on an unrelated study. Joann F. Gruber was employed as a research assistant at Merck and Co., Inc. during 2014 to research rotavirus vaccines.

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