TRANSMISSION OF NOROVIRUS WITHIN HOUSEHOLDS IN QUININDE, ECUADOR

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Abstract: We studied the transmission of norovirus infection in households in Quininde, Ecuador. Among household contacts of norovirus positive children with diarrhea, norovirus negative children with diarrhea and asymptomatic controls, infection attack rates were 33%, 8% and 18%, respectively (N = 45, 36, 83). Infection attack rates were higher when index children had a higher viral load.

Key Words: Norovirus, household transmission, viral load, Ecuador

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Noroviruses, the leading cause of acute gastroenteritis worldwide across all ages, are associated with approximately 18% of acute gastroenteritis in both low- and high-income countries.[1] Several attributes may play a role in the high incidence of noroviruses across the age range and across populations, including the amount of virus shed in stool, low infectious dose, relative stability outside the human host, great viral diversity and limited immunity.[2] Although a few studies have identified risk factors for norovirus transmission in the community (mainly contact with a person with gastrointestinal symptoms),[3–5] data on the contagiousness of norovirus and the factors affecting spread under conditions of intense exposure (eg, in household settings) are limited.[6] Improved understanding of norovirus transmission may help identify specific risk factors and target groups to optimize control strategies, including for vaccines, which are currently progressing through development.[7] Our objectives were to study the infection patterns and risk factors for transmission of norovirus within households in a periurban community in Ecuador.

MATERIALS AND METHODS

This was an analysis of a convenience sample (N = 496) of whole stool specimens originally collected during a household transmission study of rotavirus in Quinidine, Ecuador.[8] In brief, children aged <5 years presenting with and without diarrhea (defined as ≥3 liquid stools in 24 hours, lasting <14 days) were recruited at a local hospital and surrounding family clinics, from February 2011 to May 2012, and stool specimens were obtained within 48 hours. Based on rotavirus testing results, children with diarrhea were classified either as *cases* (if they tested positive) or *diarrhea controls* (if they tested negative). A second comparison group, children presenting for routine follow-up and who were asymptomatic, were classified as *healthy controls* (regardless of testing results). To study transmission within households, stool specimens were requested from child and adult household members of both cases and controls. For case and diarrhea control households, specimens were collected 5 to 9 days after the onset of diarrhea in the recruited child. For healthy controls, specimens were collected within 1 day of the household visit. All specimens were stored at −20°C. In this evaluation, a sample of available specimens from cases, diarrhea and healthy controls, and household contacts were tested for norovirus by real time reverse transcription and quantitative polymerase chain reaction,[9] and reclassified based on these results. Positive samples were genotyped by sequence analysis to determine whether family members were infected with the same type as the index child.[9]

Infection attack rates (iARs) among contacts were calculated for case and control households as the proportion of family members that tested positive for norovirus. We investigated potential risk factors for transmissibility to household contacts (based on characteristics of "index" children, ie, cases and norovirus positive healthy controls), as well as potential risk factors for susceptibility (based on characteristics of the household and household member contacts of index children). Logistic regression models were fit using robust standard errors to estimate the odds ratio of infection as a binary outcome, based on each potential transmissibility and susceptibility factor. First, bivariate models were used. Then, to account for possible confounding effects between variables, we developed multivariable regression models, including all variables with P values <0.2 in the univariable analysis. Analyses were conducted in Stata 12.0 (STATA Corp, College Station, TX).

RESULTS

Stool samples from 332 children were tested for norovirus. Of these, 186 had diarrhea, and 146 were healthy controls. Nineteen (10%) of the 186 children presenting with diarrhea and 15 (or 10%) of the 146 healthy controls tested positive for norovirus. Case, diarrhea control and healthy control children were similar in terms of month of enrolment ($P = 0.4$) and age ($P = 0.7$). Only 1 case had previously tested positive for rotavirus, and none of the healthy controls. Stool samples from 164 contacts within 52 households were tested for norovirus. iARs were highest among household contacts of cases, 33% (15/45), compared with iARs among household contacts of diarrhea controls [8% (3/36); $P < 0.01$] and healthy controls [18% (15/83); $P = 0.05$]. Among household contacts of norovirus positive and norovirus negative healthy controls, iARs were 15% (7/48) and 23% (8/35), respectively ($P = 0.3$). Cycle threshold (Ct) values were similar among cases (median 23, range 16–35, N = 14) and norovirus positive healthy controls (median 22, range 16–36, N = 14), the 2 groups comprising index children ($P = 0.8$).

The effect of index child characteristics (ie, transmissibility factors) and contact and household characteristics (ie, susceptibility factors) on iARs are shown in Table, Supplemental Digital Content 1, http://links.lww.com/INF/C182. With respect to transmissibility, iARs were nonsignificantly higher among household contacts when the index child was younger (29% from children <24 months compared with 17% from children 24 to 59 months; OR = 2.1, $P = 0.1$). iARs were significantly higher if the index child had symptoms (33% compared with 15%; OR = 2.9, $P = 0.03$) or a higher viral load (low Ct values; 31% with Ct < 23, versus 11% with a Ct ≥ 23;

TABLE 1. Genotype Profiles of Norovirus Infections Detected in Index Children and Contacts in 12 Households with ≥2 Typed Specimens

HH No.*	Index	Contact				
		1	2	3	4	5
	GT	GT	GT	GT	GT	GT
1	GI.3	GI.3				
2	GI.3	GI.3	GI.3	GI.3		
3	GII.6	GII.6	GII.6	*GII.16*		
4	GI.1	*GII.4*†				
5		GII.6	GII.6	GII.6	GII.6	GII.6
6		GI.7	GI.7	*GII.16*		
7	GII.6	GII.6	GII.6+GI.3			
8	GII.16	GII.16	GII.16			
9	*GI.3*	*GII.16*	*GII.21*			
10	GII.6	GII.6				
11	GI.3 + GII.8	GI.3				
12		GI.1	GI.1	GI.1 + GII.16		

Each row represents a household (HH). Single infections within a household are shown in normal, and mixed infections are shown in italic.

*Households numbered 1–5, 6 and 7–12, are case, diarrhea control and healthy control households, respectively.

†Yerseke strain.

GT indicates genotype.

OR = 3.7, $P = 0.02$). Regarding susceptibility, iARs were significantly higher in larger households (46% with total residents ≥6 compared with 20% with total residents <6; OR = 3.4, $P = 0.04$).

In the multivariable model, only a higher viral load in the index child remained independently associated with a higher risk of infection among household contacts.

Sixty (90%) of the 67 stool samples that tested positive for norovirus were genotyped. Seventeen (or 28%) were positive for GI, 38 (or 63%) were positive for GII, and 5 (or 8%) were positive for both GI and GII. GII.6 was the most frequently detected type (in 28% of positive specimens), followed by GI.3 (in 22%) and GII.16 (in 20%).

Table 1 shows the profiles of infections in 36 individuals within 12 households with ≥2 typed specimens. Overall, 29 (or 81%) of the 36 individuals within households were infected with an identical strain, and a common strain infected all individuals within 8 (or 67%) of the 12 households. However, there was clear evidence of circulation of multiple noroviruses within several households. In only 4 (44%) of 9 households was a single genotype found among the index child and all household contacts; there were 3 households where index children and contacts had discordant types. In addition, dual norovirus co-infections were identified in 1 index children and 2 household contacts.

DISCUSSION

Our results suggest that noroviruses are highly transmissible in household settings. Overall, about one-third of household contacts of symptomatic children showed evidence of infection. High transmissibility is supported by the overall congruence of strains within households, and the predominance of a single genotype among family members. However, in stark contrast to rotavirus transmission within households,[8] infection in diarrhea and healthy control household contacts (8% and 18%, respectively), and substantial circulation of multiple noroviruses within households, suggest considerably higher levels of background infections and asymptomatic transmission with noroviruses.

By comparing symptomatic children (cases) with asymptomatic children (norovirus-positive healthy controls), we have observed that the presence of symptoms was a strong driver of onward norovirus transmission. Similarly, lower Ct values, indicating higher volume of viral excretion, were also associated with increased attack rates. Viral load is an indicator of disease-causing norovirus infection,[10] yet Ct values remained significant even after controlling for the presence of symptoms, suggesting that levels of shedding are independently associated with transmissibility. The iARs among household contacts of symptomatic children were almost ~3-fold higher to those seen among household contacts of asymptomatic children, still, a considerable proportion (15%) of the latter were also infected. In addition, Ct values were similar among symptomatic and asymptomatic children. This suggests that symptoms may not be essential for norovirus transmission. This is consistent with the concept that while norovirus is shed at relatively lower concentrations during asymptomatic infection,[10] the estimated infectious dose for norovirus is very low.[11] In addition, a few reports have linked norovirus transmission to asymptomatic food-handlers.[12,13]

Several limitations should be considered. First, for household contacts, complete information was unavailable on symptoms, thus, we were unable to evaluate attack rates or risk factors for norovirus disease. Second, we cannot be certain that a child who presented to the hospital or the clinic was the first to be infected in the home, and discerning who infected whom was not possible. However, most of the family members were infected with identical strains, including in norovirus positive healthy control households, likely indicating transmission within households, rather than multiple introductions. Third, there was the possibility of cross-contamination of specimens within the household, as we relied on self-collected specimens. However, identified risk factors argue for a true pattern of transmission, rather than random contamination. Finally, the small sample size precluded risks factors for transmission to be detected with reasonable statistical confidence.

In conclusion, our results highlight the remarkable infectiousness of noroviruses, and elucidate the association between high fecal viral excretion and norovirus transmission. We describe other possible risk factors for increased transmission, including symptomatic infections and young age. Thus, vaccines that reduce viral excretion, that prevent symptoms, and that target infants and young children, may potentially have the greatest population impact. Future household transmission studies should investigate risk factors for symptomatic norovirus disease, and aim to understand the extent to which asymptomatic infections lead to transmission, illnesses, and overall norovirus disease burden.

REFERENCES

1. Ahmed SM, Hall AJ, Robinson AE, et al. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *Lancet Infect Dis*. 2014;14:725–730.
2. Hall AJ. Noroviruses: the perfect human pathogens? *J Infect Dis*. 2012;205:1622–1624.
3. Phillips G, Tam CC, Rodrigues LC, et al. Risk factors for symptomatic and asymptomatic norovirus infection in the community. *Epidemiol Infect*. 2011;139:1676–1686.
4. de Wit MA, Koopmans MP, van Duynhoven YT. Risk factors for norovirus, Sapporo-like virus, and group A rotavirus gastroenteritis. *Emerg Infect Dis*. 2003;9:1563–1570.
5. Karsten C, Baumgarte S, Friedrich AW, et al. Incidence and risk factors for community-acquired acute gastroenteritis in north-west Germany in 2004. *Eur J Clin Microbiol Infect Dis*. 2009;28:935–943.
6. Conrad D, Dee K, Keenan A, et al. The role of household transmission in an outbreak of viral gastroenteritis in a primary school in Liverpool, England. *Public Health*. 2013;127:882–884.
7. Atmar RL, Bernstein DI, Harro CD, et al. Norovirus vaccine against experimental human Norwalk Virus illness. *N Engl J Med*. 2011;365:2178–2187.
8. Lopman B, Vicuña Y, Salazar F, et al. Household transmission of rotavirus in a community with rotavirus vaccination in Quininde, Ecuador. *PLoS One*. 2013;8:e67763.
9. Trujillo AA, McCaustland KA, Zheng DP, et al. Use of TaqMan real-time reverse transcription-PCR for rapid detection, quantification, and typing of norovirus. *J Clin Microbiol*. 2006;44:1405–1412.
10. Phillips G, Lopman B, Tam CC, et al. Diagnosing norovirus-associated infectious intestinal disease using viral load. *BMC Infect Dis*. 2009;9:63.
11. Teunis PF, Moe CL, Liu P, et al. Norwalk virus: how infectious is it? *J Med Virol*. 2008;80:1468–1476.
12. Nicolay N, McDermott R, Kelly M, et al. Potential role of asymptomatic kitchen food handlers during a food-borne outbreak of norovirus infection, Dublin, Ireland, March 2009. *Eurosurveillance*. 2011;16:10–15.
13. Thornley CN, Hewitt J, Perumal L, et al. Multiple outbreaks of a novel norovirus GII.4 linked to an infected post-symptomatic food handler. *Epidemiol Infect*. 2013;141:1585–1597.

PERICARDIAL EFFUSION AND ADENOSINE DEAMINASE FALSE POSITIVITY DUE TO PARVOVIRUS B19

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Abstract: This case is presented to highlight that one of the causes for massive exudative pericardial effusion in a child may be parvovirus B19, and adenosine deaminase can be falsely positive in such patients.

Key Words: pericarditis, Parvovirus B19, adenosine deaminase

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Most cases of viral pericarditis occurring in childhood involve coxsackievirus, Epstein-Barr virus (EBV), influenza, adenoviruses, echoviruses, varicella, hepatitis b, mumps and measles. Parvovirus B19 can cause acute heart failure by pericarditis,[1] and glomerulonephritis,[2] and transient erythroblastopenia.[3] We present a case of a 5-year-old otherwise healthy female admitted with parvovirus B19 massive pericardial and pleural effusions, of an exudative nature and adenosine deaminase (ADA) falsely positive.

In this case report, we want to emphasize that parvovirus B19 infection causes anemia, pneumonia, pleural and pericardial massive exudative effusion with ADA false positivity which is similar to tuberculosis.

CASE

A 5-year-old female was referred to our pediatric cardiology polyclinic on detection of cardiomegaly on chest radiography. She was admitted with weakness and abdominal pain. She was conscious and cooperative. Her axillar temperature was 36.7°C, breathing rate was 30/min, pulse was 130/min, and a pericardial rub was audible. Blood pressure was 100/60 mmHg (50–90% percentile). Abdominal examination revealed a midclavicular, 4 cm palpable liver. The spleen was nonpalpable and Traube's space was open. Electrocardiography revealed low voltage and diffuse T-negativity. Transthoracic echocardiography revealed free pericardial fluid on a subcostal imaging, extending to 6 cm.

The patient was taken into the intensive care unit and was monitored. Echocardiography-guided pericardiocentesis was performed and 500 mL of hemorrhagic fluid was removed. Blood and pericardial fluid samples were sent to the laboratory for etiologic examination. In the whole blood count: total leukocyte counts = 7070/μL, hemoglobin= 8.2 g/dL, the number of platelets = 357,000/μL, erythrocyte sedimentation rate = 13 mm/h, C reactive protein was negative (3 mg/dL), prothrombin time = 12″, prothrombin activity = 97%, INR = 1 and activated partial thromboplastin time = 32. Biochemistry from blood and pericardial fluid (total protein, glucose, LDH), and cell count, culture and ADA level from pericardial fluid (blood: glucose: 65 mg/dL, LDH: 594 U/L, protein:7 g/dL, pericardial fluid: glucose: 25.8 mg/dL, LDH: 2831 U/L, protein: 5305 mg/dL, glucose fluid/blood = 0.38, protein fluid/blood = 0.7, LDH fluid/blood = 4.7) indicated that the fluid was exudative.

Cellular examination of the fluid revealed 65% lymphocyte. The ADA value was 76.6 U/L. As there was no respiratory distress or worsening of the overall condition of the patient, nonspecific antibiotic (ampicillin sulbactam) and anti-inflammatory (ibuprofen) treatment was initiated and monitored. For acid-fast bacteria (ARB) testing, 3-day fasting gastric fluid was sent for analysis which was negative. PPD skin test was negative. Thorax computed tomography revealed thickening in the left lung fissure, and a consolidation area with air bronchogram in the lower lobe of the left lung. There was pleural effusion (left pleura: 3 cm) in both lungs.

There was no growth in the nonspecific cultures or *Mycobacterium tuberculosis* cultures and pleural fluid. EBV-VCA IgM, IgG, anti-HIV, *Mycoplasma pneumonia* IgM and IgG antibodies were also negative in serum. Parvovirus B 19 IgM was 8.1 (+), IgG was 3.83 (+), and parvovirus B19 PCR was 4800 IU/mL. Serum ferritin of 39.81 ng/mL, folic acid of 10.46 ng/mL, and vitamin B12 of 221.2 pg/mL indicated anemia. There was no finding of hemolysis and no blasts were observed on a peripheral smear. The fecal occult blood test was negative. It was considered that the decreased series of erythroid might be associated with parvovirus B19 infection. There was decreased pericardial fluid after 3 days and the patient was discharged with only anti-inflammatory treatment, weekly echocardiography. On the follow-up echocardiography and thorax US after 4 weeks, pericardial and pleural fluid had resolved. The hemogram revealed that Hgb had increased to 10.6 g/dL. Parvovirus B 19 IgM was (−), IgG was (+), ADA was 17 U/L, which was checked again 3 months later.

DISCUSSION

ADA estimation in pleural fluid has long been a marker for tuberculous pleurisy. Values above 40 U/L indicate pleural