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Research paper

Pediatric norovirus GII.4 infections in Nicaragua, 1999–2015

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ABSTRACT

Objectives: Investigate clinical and epidemiological factors of pediatric GII.4 norovirus infections in children with acute gastroenteritis (AGE) in Nicaragua between 1999 and 2015.

Methods: We retrospectively analyzed laboratory and epidemiologic data from 1,790 children ≤ 7 years with AGE from 6 hospitals in Nicaragua (n = 538), and 3 community clinics (n = 919) and households (n = 333) in León, between 1999 and 2015. Moreover, asymptomatic children from community clinics (n = 162) and households (n = 105) were enrolled. Norovirus was detected by real-time PCR and genotyped by sequencing the N-terminal and shell region of the capsid gene.

Results: Norovirus was found in 19% (n = 338) and 12% (n = 32) of children with and without AGE, respectively. In total, 20 genotypes including a tentatively new genotype were detected. Among children with AGE, the most common genotypes were GII.4 (53%), GII.14 (7%), GII.3 (6%) and GI.3 (6%). In contrast, only one (1.4%) GII.4 was found in asymptomatic children. The prevalence of GII.4 infections was significantly higher in children between 7 and 12 months of age. The prevalence of GII.4 was lowest in households (38%), followed by community clinics (50%) and hospitals (75%). Several different GII.4 variants were detected and their emergence followed the global temporal trend.

Conclusions: Overall our study found the predominance of pediatric GII.4 norovirus infections in Nicaragua mostly occurring in children between 7 and 12 months of age, implicating GII.4 as the main norovirus vaccine target.

1. Introduction

Globally, norovirus is a key pathogen associated with nearly a fifth of all cases of acute gastroenteritis [Ahmed et al., 2014]. In countries where infant vaccination has reduced the incidence of rotavirus disease, norovirus has become the most common cause of pediatric gastroenteritis [Bucardo et al., 2014; Payne et al., 2013]. Despite efforts to understand norovirus disease epidemiology in low- and middle-income countries (LMIC), there is still limited comprehensive epidemiological studies from these areas [Ayukekbong et al., 2015; da Silva et al., 2016]. A recent review and meta-analysis from Latin American studies showed a norovirus prevalence in acute gastroenteritis (AGE) cases of 15% in the community, 14% in outpatient settings, 16% in hospital locations and 8% among asymptomatic subjects [O'Ryan et al., 2017].

Viruses belonging to the *Norovirus* genus in the *Caliciviridae* family can be divided into at least six genogroups (GI to GVI), of which GII viruses cause the majority (> 80%) of disease in humans and GI viruses being less common detected(< 11%) [Pringle et al., 2015; Vinje, 2015]. By phylogenetic analysis of the major capsid protein VP1, 22 genotypes have been recognized in GII and 9 in GI [Kroneman et al., 2013; Vinje, 2015]. Among the GII genotypes, GII.4 is the single most common genotype infecting humans worldwide, associated with approximately 60% of all reported norovirus outbreaks [Siebenga et al., 2009] and 70% of sporadic norovirus gastroenteritis in children [Hoa Tran et al., 2013].

Since 1995, six different GII.4 pandemic variants have emerged and each of them replaced a previous predominant GII.4 variant. Studies suggest that the antigenic changes in the new GII.4 variants result both

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in loss of neutralizing epitopes as well as changing ligand in the host over time, both which may contribute to escape of herd immunity [Lindesmith et al., 2012]. In late 1995, GII.4 US95_96 emerged causing the first reported norovirus pandemic [Fankhauser et al., 2002]. In 2002, GII.4 Farmington Hills viruses emerged followed by GII.4 Hunter in 2004–2005 in Australia, Europe, Asia and Central America [Bucardo et al., 2008; Bull et al., 2006; Kroneman et al., 2006; Lopman et al., 2004; Phan et al., 2006]. In 2006, GII.4 Den Haag emerged and became the predominant variant [CDC, 2007; Kroneman et al., 2006; Siebenga et al., 2008] followed by GII.4 New Orleans in 2009 which gradually replaced GII.4 Den Haag viruses [Vinje, 2015]. In 2012, GII.4 Sydney viruses emerged and became pandemic [Hoa Tran et al., 2013].

The reason why GII.4 is the dominant genotype worldwide is unclear, but a combination of host factors and increased virulence of viruses belonging to this genotype have been suggested. GII.4 noroviruses infect secretor positive individuals, which express α1,2-linked fucose residue on surface epithelial cells of the gut and in body fluids [Le Pendu et al., 2006]. Secretors represent ≥ 80% of several populations worldwide, whereas other genotypes could have a more narrow host specificity [Nordgren et al., 2016]. GII.4 strains are undergoing antigenic variation at a faster rate as compared to other genotypes, likely in response to herd immunity [Bull et al., 2010; Debbink et al., 2013; Lindesmith et al., 2012; Lindesmith et al., 2011]. Moreover, observations of increased viral shedding in children infected with GII.4 compared to other genotypes, suggest that GII.4 genotypes could have higher replication rates and thus higher transmission between individuals [Bucardo et al., 2008].

This study aims to demonstrate the long term predominance and clinical severity of pediatric gastroenteritis associated with one particular norovirus genotype (GII.4), by obtaining new genotypes data of archived specimens and complementing with genotypes data of studies published elsewhere [Bucardo et al., 2011; Bucardo et al., 2010; Bucardo et al., 2008].

2. Material and methods

2.1. Subjects and specimens

We retrospectively analyzed laboratory and epidemiologic data from 1790 children ≤ 7 years (median = 14; IQR = 8–25) with AGE from 6 hospitals in Nicaragua (n = 538), and 3 community clinics (n = 919) and households (n = 333) in León, between 1999 and 2015. Moreover, 267 asymptomatic children (median age = 18; IQR = 5–30) from community clinics (n = 162) and households (n = 105) were also enrolled. Setting data and samples were not consistently available from every year, thus, material from the hospital represent 2002–2013 and from the community 1999–2010 and 2015, with exception of 2007 and 2008 for both settings. Household represents 2010–2011 and asymptomatic 2005–2006, 2010–2011. All children with AGE were clinically evaluated by pediatricians or general practitioners following the World Health Organization (WHO) strategy for diarrhea management. Accordingly, only children with signs of dehydration and not tolerating oral rehydration were treated at the hospital, otherwise they were treated at the community clinics in the oral rehydration unit. Sampling at the hospital was performed within 24 h after admission. All samples were collected following a standard procedure that involved collection in sterile plastic containers and transportation at 4 °C to the laboratory of Microbiology and the Faculty of Medical Science of UNAN-León. A 10% (wt/vol) suspension of the stools was prepared with phosphate-buffered saline (pH = 7.2), and two aliquots were frozen at − 20 °C for virus analysis.

2.2. RNA extraction and reverse transcription

Viral RNA was extracted from 200 µl of 1:10 stool suspensions using High Pure Viral RNA Kit (Roche Diagnostics) following the manufacturer's instructions. A total of 50 µl of RNA was collected and stored at − 20 °C until reverse transcription. Reverse transcription (RT) was carried out as described previously and cDNA was stored at − 20 °C until used [Bucardo et al., 2008]. RNA from household and asymptomatic samples from 2010 and 2011 were purified by using automated RNA purification (either EZ1 robot (QIAGEN) or KingFisher magnetic particle processor (Thermo Fisher Scientific).

2.3. Real-time PCR assays for detection of norovirus

SYBR real-time RT-PCR was used for norovirus screening of samples collected at hospital and community between 2009 and 2013 and 2015 from symptomatic children. In brief, 2.5 µl of cDNA was added to a reaction mixture consisting of 12.5 µl of FastStart Universal SYBR Green Master (ROX) (Roche Applied Science, IN, USA), 0.4 pmol of each GII primers (NVG2f2 and COG2R) or each GI primers (NVGIF1b and NVG1r) [Nordgren et al., 2008], and 8 µl of RNAse free water, to final volume of 25 µl. The real-time PCR reactions were performed in a 96-well reaction plate using the ABI 7500 Real Time PCR System (Applied Biosystems, Foster, CA). PCR was performed under the following conditions: 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s, 55 °C for 30 s and 72 °C for 1 min. 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 Tm of 76.1 ± 0.6 °C for GI and 77.1 ± 0.6 °C for GII. A Taqman assay previously described by Nordgren and coworkers [Nordgren et al., 2013] was used for norovirus screening of samples collected at hospital and community between 1999 and 2006 from symptomatic and asymptomatic children. Household and asymptomatic samples from 2010 and 2011 were analyzed by using the TaqMan assay previously described Kageyama and coworkers [Kageyama et al., 2003].

2.4. Norovirus genotyping

Nucleotide sequencing of the N-terminal and shell (NS) region of the capsid gene was performed by Macrogen Inc. (Seoul, South Korea). The sequencing reaction was based on BigDye chemistry; NVG1f1b or NVG2f2 forward primers and G1SKR or G2SKR reverse primers were used as sequencing primers for norovirus GI (381 bp) or GII (378 bp), respectively [Kojima et al., 2002; Nordgren et al., 2008]. Genotypes were determined in norovirus positive samples by submitting the sequences to either the Norovirus Genotyping Tool Version 1.0 or to CaliciNet, both systems can determine genotype or GII.4 variant upon analyzing the NS sequences [Kroneman et al., 2011; Vega et al., 2011].

2.5. Statistical analysis

We first generated descriptive statistics to characterize norovirus infections by genotypes and GII.4 variants, stratified by setting and year of collection. We then estimated odds ratios (OR) and 95% confidence intervals (CI) to investigate the associations between GII.4 infections and setting, age group, and quarter using logistic regression. Adjusted Odds ratios and 95% CIs (variables setting, age group, and quarter) was calculated using multivariable logistic regression in SPSS. Statistical significance of temporal peaks of norovirus incidence was determined by logistic multivariate analysis of norovirus incidence by bimester. An alpha of 0.05 was used to determine statistical significance. All analyses were performed using SPSS (Statistical Program for Social Science version 14.0.0 for Windows; Chicago, IL).

3. Results

3.1. One fifth of pediatric AGE in Nicaragua was associated with norovirus

A total of 338 (19%) of the 1790 symptomatic and 32 (12%) of the