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Brief Original Article

Detection of rotavirus and norovirus among children with acute gastroenteritis in Merida and Chihuahua cities, Mexico

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Abstract

Introduction: Infantile acute gastroenteritis (AGE) is a leading cause of morbidity and mortality, particularly in developing countries. The most frequent etiological agents of viral gastroenteritis in children are adenovirus, astrovirus, rotavirus, and norovirus, the last two, leading causes. Thus, the aim of this study was to identify the presence of these two viruses in children with AGE, from two cities located in the Southeast and the Northwest regions of México.

Methodology: HuNoVs were detected and characterized by RT-PCR and sequencing, while RVs were detected by RNA electrophoresis.

Results: The presence of RV and HuNoV was evaluated in 81 stool samples; 37 were collected between April and July 2013 from patients with acute diarrhea in Merida, and 44 were collected between January and June 2017 in Chihuahua, who attended health services. Despite vaccination, RV resulted in the predominant viruses detected, with 30.8% (25/81) positivity, while HuNoV infection was present in 8.6% (7/81) of the stool samples; GII strains were identified circulating in the Southeast, while GI strains were identified in the Northwest. Moreover, co-infections with both viruses were detected at a prevalence rate of 2.4% (2/81).

Conclusions: The circulation of RV and HuNoV in the country is continuous and should be constantly monitored due to their impact on public health.

Key words: Rotavirus; norovirus; gastroenteritis; Mexico.

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Introduction

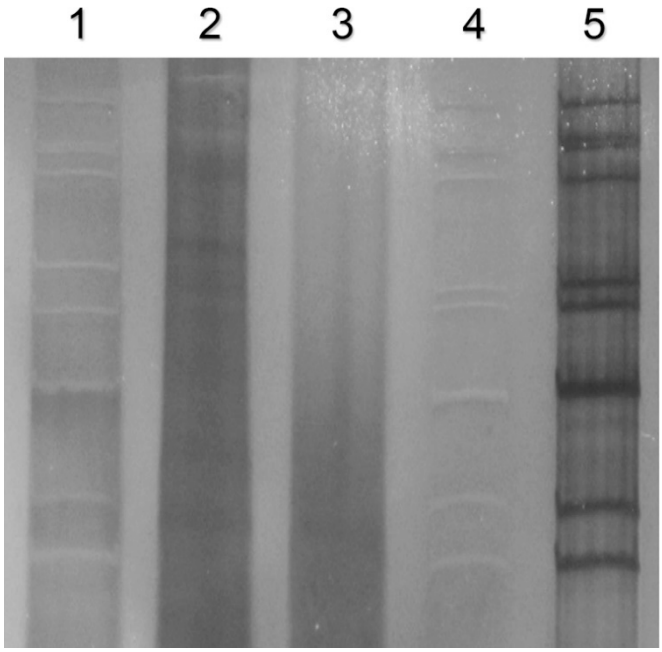
Diarrheal disease is the second cause of death for children under the age of five, killing 525,000 children each year. Viral infections represent the main cause of infantile acute gastroenteritis (AGE) [1], and are caused by astroviruses, adenoviruses, rotaviruses, and noroviruses, being the last two leading causes. Other agents such as coronavirus, aichivirus, parvovirus, pestivirus, and enterovirus have also been associated with AGE. While adenoviruses cause between 2 and 3.6% of diarrhea episodes, 4.7% of hospitalizations, and 3.1% of deaths from this sickness worldwide, in children under 60 months, astroviruses cause between 2.3 to 4% of diarrhea episodes, 3% of hospitalizations and 2.1% of deaths from this sickness in this same group of age [2].

Rotavirus group A (RVA) is the major cause of AGE in young children worldwide. It belongs to *Rotavirus* genus in the *Reoviridae* family, consisting of icosahedral viruses with genomes from 9-12 segments of double-stranded RNA. RV is transmitted following a fecal-oral route; the seasonality of infection varies among countries and is influenced by the climate from one region to another [3]. Despite the availability of efficient vaccines, RVA continues to be the major cause of acute gastroenteritis (AGE) in young children, mainly in developing countries. The change in the circulating strains due to the selective vaccine pressure

or to the natural diversity fluctuation has been recently analyzed for Mexico and other countries from Latin America, Europe, Africa, and Australia [4]. In Mexico, 70% of children hospitalized for diarrhea are of viral etiology, predominantly by RV [5]; yet, after the introduction of the RV vaccines, a significant reduction in mortality, hospitalizations, and new cases has been observed. Because RV is still the leading viral gastrointestinal disease, it is important to maintain surveillance and official reports of positive cases [5].

Human norovirus (HuNoV), member of the *Caliciviridae* family, is the leading cause of AGE, both in adults and children worldwide, particularly from countries with successful RV vaccination campaigns [6]. In developing countries, its prevalence is underestimated because a specific diagnosis is not usually made. HuNoV may cause 1 million hospitalizations and 200,000 deaths of children less than 5 years of age annually [7]. The *Caliciviridae* family is composed of positive-sense RNA viruses, classified into eleven genera, including *Norovirus* and *Sapovirus,* that comprise the caliciviruses that infect humans, also known as Human caliciviruses (HuCVs) [8]. Members of the *Norovirus* genera are classified into seven genogroups (GI-GVII), containing numerous genotypes. HuNoV illnesses and outbreaks are usually more common from winter to spring seasons, affecting low and high-income countries, transmitted through food and contaminated water. In Mexico, the HuNoV incidence as single etiologic agents in sporadic cases of AGE is about 5-7% [9], and a high prevalence of antibodies to HuNoV and asymptomatic cases have been reported in children under 5 years of age [10]. However, since the HuNoV diagnosis is not conducted as routine surveillance, the available numbers most likely do not reflect the general country situation [11,12]. Here, we analyzed the presence of RV and HuNoV from stool samples randomly collected in two distant cities in different years and seasonal periods from children with AGE. Despite the random and differences in the sampling times of the analyzed samples, the constant circulation of these viruses as causative agents of AGE was evidenced, which highlights the importance of establishing systematic surveillance that reflects the situation throughout the country.

Figure 1. Electropherogram of rotavirus strains isolated from stool samples isolated in Mexico. The genomic RNA 1-11 segments migration pattern of positive samples were analyzed in a 7.5% polyacrylamide gel stained with silver stain. Lanes 1 and 4: RV positive stool controls. Lanes 2 and 3: RV negative stool controls. Lane 5: RV-positive stool sample showing the migration pattern typical of a group A rotavirus.



Methodology

Diarrheic samples from children (6 months to 13 years) who attended two public health care units: Hospital de la Amistad Corea-México in Merida and Hospital Infantil de Especialidades de Chihuahua, were collected between April and July 2013 (n = 37) and between January and June 2017 (n = 44), respectively, and stored at -80 °C until used. AGE was defined as at least three liquid stools/24h. Information on age and gender was obtained from the clinical history. Informed consent was obtained from the parents, and the protocol was approved by the Internal Review Board of each institution (COBISH-028/2016).

Viral RNA was extracted from fecal suspensions (10% w/v in phosphate buffer saline) using Trizol reagent (Gibco BRL, Gaithersburg, MD. Cat. No. 18068). For RV detection, viral RNA was analyzed on a 7.5% PAGE and silver stained as described [13] and (Figure 1). HuNoV screening was performed by RT-PCR as previously described [14], using primers 289H and 289I [15] and 290YM [14]. Amplicons of 319 bp corresponding to the RNA-dependent-RNA-polymerase (RdRp) gene were analyzed by agarose gel electrophoresis, treated with DNA clean and concentrator S^{TM} Kit (Zymo Research, Orange, CA. Cat. No. D7022) and sequenced in both directions at the DNA Synthesis and Sequencing Unit, Instituto de Biotecnología (IBT), Universidad Nacional Autónoma de México (UNAM). Sequence identity was analyzed by BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and the

evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method. Ambiguous positions were removed for each sequence pair. Evolutionary analyses were conducted in MEGA software version X. HuNoV sequences were deposited in the GenBank (NCBI) under the following accession numbers: Merida samples: MZ064635, MZ064636, MZ064637, MZ064638, MZ064639; Chihuahua samples: MZ063688, MZ063689.

Results

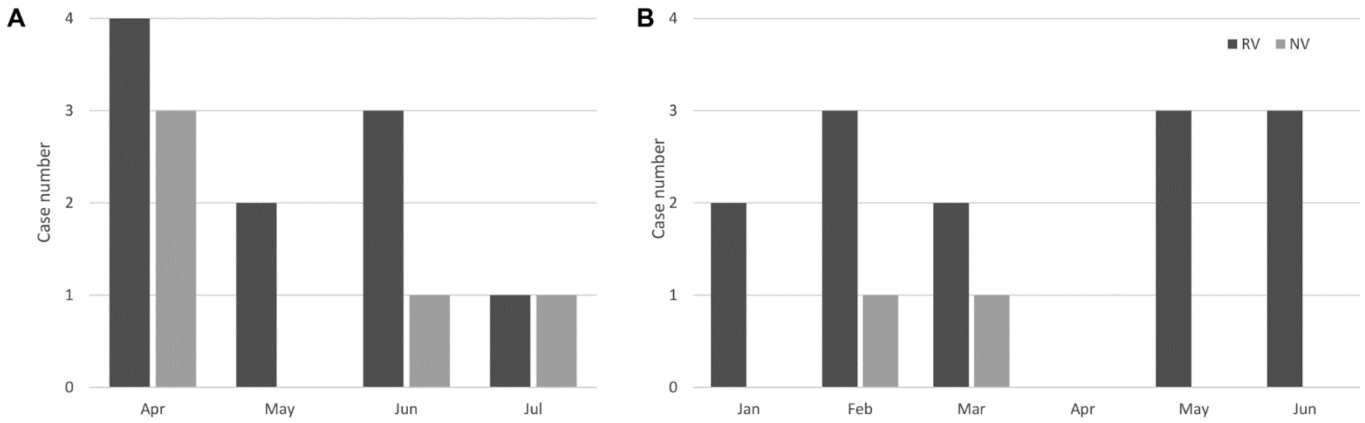
RV was detected in 30.8% (25/81) and HuNoV in 8.6% (7/81) of the total samples (Table 1). The frequency of RV and HuNoV in samples from Merida was 27% (10/37) and 13.5% (5/37), respectively; one coinfection, representing 2.7% (1/37), was detected. A higher occurrence of HuNoV and RV in females was observed. The frequency of RV and HuNoV from Chihuahua was 34% (15/44) and 4.5% (2/44), respectively; one coinfection, representing 4.4% (1/44), was detected. Due to the low number of positive samples, differences in gender frequency could not be determined. The number of positive cases of RV from Merida and Chihuahua was: 80 and 33.33%, respectively, in children younger than 24 months of age and 20 and 66.66%, respectively, in children older than 24 months of age. The number of positive cases of HuNoV from Merida and Chihuahua was: 60 and 50%, respectively, in children younger than 24 months of age and 40 and 50%, respectively, in children older than 24 months of age (Table 1). RV-positive samples were distributed along April, May, June, and July (all tested periods) in Merida and in January, February, March, May, and June in Chihuahua. HuNoV positive samples were distributed along April, June, and July in Merida, where the temperature varies between 18-36 ºC during the year. In contrast, HuNoV positivity was limited to February and March in Chihuahua, a period with temperatures from 2-19 ºC, while warmer temperatures from 25 to 35ºC are reached during summer and autumn (Figure 2).

Table 1. Distribution of RV and HuNoV positive samples.

	Merida	**Chihuahua**
Total number of samples	37	44
RV infection	10 (27%)	15 (34%)
Gender		
Male	4 (40%)	5 (33.33%)
Female	6 (60%)	10 (66.66%)
Age		
< 24 Months	8 (80%)	5 (33.33%)
> 24 Months	2 (20%)	10 (66.66%)
HuNoV infection	5 (13.5%)	2 (4.5%)
Gender		
Male	1 (20%)	1 (50%)
Female	3 (60%)	1 (50%)
Non determined	1 (20%)	0
Age		
< 24 Months	3 (60%)	1 (50%)
> 24 Months	2 (40%)	1 (50%)
Coinfections	1 (2.7%)	1 (24.4%)

To characterize the HuNoV strains detected, all amplicons were subjected to direct sequencing. The nucleotide BLAST analysis (NCBI) confirmed that amplicons corresponded to the RdRp gene. The HuNoV isolates from Merida samples were classified as GII.P23 (1/5), GII.P4 (2/5), and GII.P7 (2/5). The HuNoV isolates from Chihuahua samples belong to GI.P2 (2/2). The phylogenetic analysis (Figure 3) showed two major groups corresponding to GII and GI isolates with their respective reference sequence. Isolates MZ064638 and MZ0646396 (GII group) are genetically closer with GII.P7 reference strains; isolates MZ064636, MZ064637 share the location in the same subgroup with GII.P4 reference strains and MZ064635 isolate showing the higher genetic distance belongs to

Figure 2. Monthly distribution of positive samples for RV and HuNoV in A) Merida (2013) and B) Chihuahua (2017) cities.

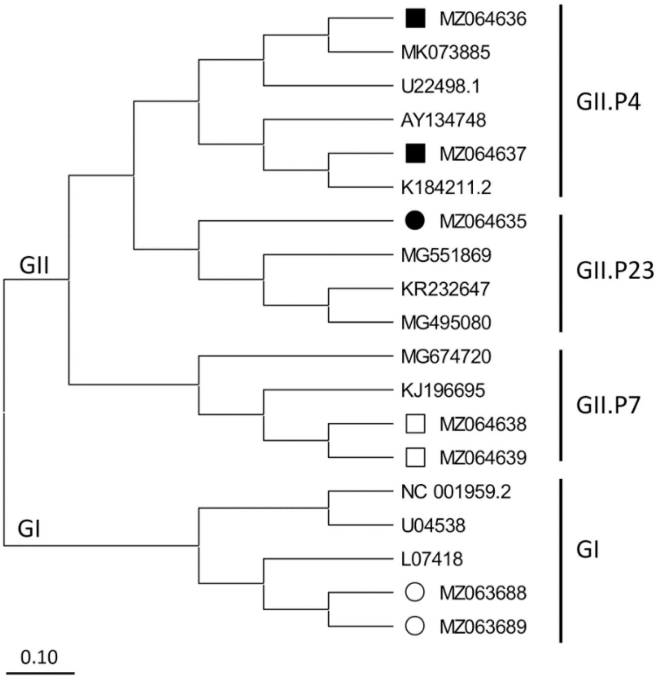


GII.P23 genotype. The isolates MZ063688 and MZ063689 share the same clade in the group GI, evidencing the high homology between them.

Discussion

Viral-induced diarrheal diseases are highly frequent in Mexico; however, the data on RV and HuNoV behavior is scarce [5]. Thus, to contribute to the knowledge of the incidence of RV and HuNoV cases after the introduction of the RV vaccine and to identify the HuNoV strains circulating in northern and southern areas of Mexico, stool samples randomly collected in two distant cities in different years and seasonal periods from children with AGE were analyzed. Despite the differences in the sampling times and the great geographical distance between Merida and Chihuahua cities, our results indicate the constant circulation of RV and HuNoV among children with a similar frequency reported from other regional and foreign studies [16]. The genetic diversity of RV and the evolution of new genotypes influences the efficacy of current vaccines; thus, their monitoring, and case notifications are particularly important. All HuNoV isolates from Merida were exclusively GII and those from Chihuahua were exclusively GI. Isolates MZ064636 and MZ064637 share a genetic cluster with the reference strain GII.P4 Sydney (MK073885), the main cause of HuNoV outbreaks worldwide since 2002 [6] . Isolates MZ064638 and MZ064639 were identified as HuNoV GII.P7, detected throughout the world in the past 5 years [17], and recently associated with outbreaks in Brazil, Australia, and China [17,18]. In a very recent study, the global and regional circulation trends of HuNoV genotypes have been analyzed based on the sequences available in public repositories of noroviruses circulating from 1995 to 2019, and the genotypes circulating, and predominance at the global and regional level have been described. GII.P4 represent the predominant circulating type, and GII.P7 is among the top 10 most frequent types [19]. The identified GII.P23 isolate clustered with a group of non-typeable strains (recently classified as GII.P23–GII.P27) circulating in South America [20], highlighting the global dissemination of HuNoVs. Isolates MZ063688 and MZ063689 from Chihuahua, clustered with GI reference strains; its lower frequency in comparison with GII observed is in accordance with previous reports in Mexico and other countries [9,10,16]. These results suggest that HuNoV strains circulating in Merida and Chihuahua are introduced from different origins.

Figure 3. Phylogenetic analysis of HuNoV recovered from Merida and Chihuahua cities. Based on a 319 bp fragment of the genomic RNA-dependent RNA polymerase region. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method. This analysis involved 19 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGA X. The bar at the bottom provides the scale of these branch lengths. 5 HuNoV from Merida belong to HuNoV GII (MZ064635, MZ064636, MZ064637, MZ064638, MZ064639) and 2 from Chihuahua belong to HuNoV GI (MZ063688, MZ063689). Different symbols (black and white circles and squares) were used to highlight the 7 samples analyzed in this work. Sequences sharing the same symbols are genetically closer each other.



Depending on the country and the surveyed population, the different prevalence of RV and HuNoV has been reported. We were able to test samples from Merida in a period from April to July, which correspond to the spring and summer, and from January to June in Chihuahua, corresponding to winter, spring, and summer. We found a uniform distribution of RV along the tested periods in both Merida and Chihuahua cities and of HuNoV in Merida but concentrated in the first trimester in Chihuahua. The high prevalence of RV and HuNoV encompasses the winter and spring periods in regions with seasonality, such as Chihuahua; however, both viruses were detected throughout the year in regions with no seasonality, such as Merida. A higher number of samples collected during a whole year need to be analyzed to establish RV and HuNoV seasonal patterns in these cities. Locally specific variables such as climatological, biological, environmental, and

behavioral that regulate transmission, virulence and persistence of viruses in host populations must be considered to predict RV and HuNoV seasonality [21,22]. Year-round incidence of these viruses in tropical areas may influence typical seasonality patterns of geographically close regions. This could explain increased RV and HuNoV cases outside of the winter period that occurs in Mexico and other countries.

The identification and notification of the viral agents causing AGE may help to predict the occurrence of new variants and contribute reducing the indiscriminate antibiotic prescription in patients with viral diarrhea [23]. This study highlights the importance of human RV and HuNoV as etiologic diarrheal agents. However, there is still a gap because of the high amount of undiagnosed AGE cases. The establishment of a robust surveillance system for viral AGE across the country would help to field this gap and to control the management of this acute pathology better.

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