Environmental Research 138 (2015) 409–415

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Rotavirus seasonality in urban sewage from Argentina: Effect of meteorological variables on the viral load and the genetic diversity

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ARTICLE INFO

Article history:
Received 17 November 2014
Received in revised form
5 February 2015
Accepted 8 March 2015
Available online 14 March 2015

Keywords:
Rotavirus
Seasonality
Sewage
Environmental monitoring
Meteorological variables

ABSTRACT

In Argentina, the rotavirus disease exhibits seasonal variations, being most prevalent in the fall and winter months. To deepen the understanding of rotavirus seasonality in our community, the influence of meteorological factors on the rotavirus load and the genetic diversity in urban raw sewage from Córdoba city, Argentina were evaluated. Wastewater samples were collected monthly during a three-year study period and viral particles were concentrated by polyethylene glycol precipitation. RT-nested PCR was applied for rotavirus detection, and VP7/VP4 characterization and real-time PCR for rotavirus quantification. Both molecular techniques showed relatively similar sensitivity rates and revealed rotavirus presence in urban wastewater in cold and warm seasons, indicating its circulation in the local community all year round. However, a slight trend for rotavirus circulation was noted by real-time PCR in the fall and winter seasons, showing a significantly higher peak of rotavirus concentration at mean temperatures lower than 18 °C and also higher, although not statistically different during drier weather. VP7 and VP4 gene characterization showed that G1 and P[8] genotypes were dominant, and temporal variations in genotype distribution were not observed. Rotavirus spread is complex and our results point out that weather factors alone cannot explain the seasonal quantitative pattern of the rotavirus disease. Therefore, alternative transmission routes, changes in human behavior and susceptibility, and the stability and survivability of the virus might all together contribute to the seasonality of rotavirus. The results obtained here provide evidence regarding the dynamics of rotavirus circulation and maintenance in Argentina.

1. Introduction

Rotavirus infections occur repeatedly in humans from birth to old age. Infections in infants and young children can result in severe diarrhea leading to dehydration, hospitalization and in some cases death, more commonly in primary infection. The outcome of rotavirus infection is more serious in developing countries where an estimated 527,000 (475,000–580,000) rotavirus-associated deaths occur annually (Parashar et al., 2009). Based on national data for Argentina, rotavirus causes marked winter seasonal peaks of gastroenteritis, when up to half of the hospitalized children with diarrhea are found positive for rotavirus. This data shows that the rotavirus disease burden in Argentine children is extensive and seasonal (Bok et al., 2001).

The rotavirus genome consists of 11 double-stranded RNA gene segments that encode six structural (VP1–VP4, VP6 and VP7) and six nonstructural (NSP1–NSP6) viral proteins (Estes, 2001). Genotypes are classified according to a binary system through the characterization of the two outer capsid proteins that are the targets of neutralizing antibodies, VP7 (G genotypes) and VP4 (P genotypes) (Kapikian et al., 2001). Worldwide, the most common genotypes associated with human infection are G1–G4 and G9, associated with P[4] and P[8] (Dennehy, 2008; Ursu et al., 2009).

Two safety and efficient rotavirus oral vaccines have been licensed in many countries since 2006 (Ruiz-Palacios et al., 2006; Vesikari et al., 2006). Both vaccines are available in Argentina, and their incorporation in the National Immunization Program is under consideration for the next years. Thereby, the rotavirus circulation pattern is driven by the natural history of wild type

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http://dx.doi.org/10.1016/j.envres.2015.03.004
0013-9351/

rotavirus infection in Argentina.

Seasonal fluctuations in the rotavirus infection are well documented (Cook et al., 1990; Levy et al., 2009; Jagai et al., 2012). Rotavirus exhibits distinct seasonality, being considered a winter disease in some parts of the world. The core of seasonality in infectious diseases is thought to be related to temporal oscillations in the governing transmission cycles of pathogenic agents and host susceptibility. Simple transmission models demonstrate that small seasonal changes in host or pathogen factors may be sufficient to create large seasonal surges in the disease incidence, which may be important, particularly in the context of global climate change (Pitzer et al., 2009).

Despite the growing attention to rotavirus disease seasonality, a solid theoretical underpinning for rotavirus seasonal peaks is limited. Observational studies of human rotavirus disease have suggested that lower temperature, lower relative humidity and lower levels of rainfall are associated with an increased risk of rotavirus disease (Jagai et al., 2012; D'Souza et al., 2008). Recently, the influence of birth rates and transmission routes has also been suggested to be involved in the seasonality of rotavirus incidence (Atchison et al., 2009; Pitzer et al., 2011). So far, the intensity of viral circulation in a community in relationship to the rotavirus disease pattern has not been studied.

The infected population excretes high numbers of rotavirus particles in feces which in turn are discharged in sewage, which is transported to wastewater treatment plants. Thus, raw sewage is likely to contain pathogenic organisms similar to those in the original human excreta (Meleg et al., 2008; Kargar et al., 2009; Mueller et al., 2009; Barril et al., 2010). In that way, the detection and quantification of the viral load in untreated wastewater could mirror the intensity and genotype diversity of rotavirus circulating in the community.

The objective of this study was to determine the influence of meteorological local factors on the viral load and the genetic diversity of human rotaviruses in urban raw sewage in Córdoba city, Argentina. This new approach can provide evidence regarding the dynamics of rotavirus circulation and maintenance in our community as well as to assess if the seasonal intensity of rotavirus circulation could explain, at least in part, the viral disease pattern documented for Argentina. This information would be important for increasing our understanding of the local epidemiology of rotavirus disease.

2. Materials and methods

2.1. Background

Córdoba city (approximately 1,330,023 inhabitants) is the capital of Córdoba province, located in the central region of Argentina. It has average monthly temperatures of around 23 °C during November–April and around 14 °C during July–September, and rainfall peaks during January through April with around 100–300 mm. The estimated per capita gross domestic product for 2012 in Argentina was 17,917 international dollars, the mortality rate in children under five years old in 2012 was 14 per thousand live births, and the total fertility rate in 2011 was 2.2. In the 2010 census, around 5.6 of the population in the province of Córdoba was reported to be living with unsatisfied basic needs (indicator based on sanitary and housing conditions, school attendance, and subsistence capacity), while 97.6% of the population in the city of Córdoba has access to drinking water. In Córdoba city, it was estimated that 1 in every 27 children in the 0–35 month-old cohort/range is annually hospitalized for a viral gastroenteritis illness. The major impact on viral diarrhea lies on the rotavirus infection, accounting for 84.0% of the viral diarrheal cases analyzed and for approximately one third of severe diarrheas requiring hospital admission in Córdoba city, Argentina.

2.2. Sample collection

Raw sewage water samples (1.5 L each) were collected from the municipal wastewater treatment plant (WWTP) named Bajo Grande in Córdoba city, Argentina. The sewerage system has a population coverage of 61% and no industrial wastewater is treated in this facility. Treated sewage water is totally discharged in the Suquía River. Sampling was carried out monthly, from February 2009 to December 2011, obtaining a total of 35 inflow samples. A systematic sampling was carried out every Tuesday of each month between 9 and 11 a.m., in order to minimize the effects of diurnal variations. Samples were randomly collected from the same sampling point, which is the inlet channel. Samples were kept in sterile containers at 4–8 °C until delivered to the laboratory, where they were processed within 24 h.

2.3. Meteorological data

Data on weather variables was obtained from the Argentine National Meteorological Service. Maximum, minimum and mean environmental temperature (°C) and relative humidity (%) were daily obtained in the 5-day period up to the wastewater sample collection. Data of cumulative precipitation (mm) was obtained on the day of sampling corresponding to the cumulative rainfall of the last 30 days. Environmental variables were classified in dry season (DS) corresponding to the period from April to September, and wet season (WS) corresponding to the period from October to March.

2.4. Sample concentration

Concentration of viruses in sewage specimens was performed using the method of polyethylene glycol precipitation previously described by Lewis and Metcalf (1988), Greening et al. (2002) and modified by Huang et al. (2005). Briefly, the 1.5 L wastewater samples were concentrated 100-fold to 15 ml by high-speed centrifugation, elution and polyethylene glycol 6000 precipitation.

2.5. Nucleic acids extraction and cDNA synthesis

Viral RNA was extracted from 140 μL of the concentrated sample using the commercial QIAmp Viral RNA kit (Qiagen Inc., Hilden, Germany). The manufacturer's protocol was followed, and the purified viral RNA was eluted in 60 μL of elution buffer. Extracted RNA was reverse-transcribed into cDNA using random hexamer primers and AMV reverse transcriptase (Invitrogen, CA, USA).

2.6. Rotavirus G and P genotyping

cDNA products were used as templates for PCR VP7 gene amplification with the Beg9/End9 pair of primers (Gouvea et al., 1990) and VP4 gene amplification with the Con2/Con3 primers (Gentsch et al., 1992). G and P typing methods were used as described previously (Gouvea et al., 1990; Gentsch et al., 1992; Iturriza-Gomara et al., 2000). Briefly, multiplex nested PCR was carried out separately for the VP7 and VP4 genes using the amplified products of the first RT-PCR of VP7 and VP4 genes as templates. For G and P genotyping G1 to G4, G8, G9 and P[4], P[6], P[8], P[9], P[10]-specific primers were used in the multiplex nested PCRs, respectively. The amplicons were analyzed by electrophoresis on 10% polyacrylamide gels and visualized after silver staining, as described elsewhere (Herring et al., 1982), to achieve high resolution of the products obtained.