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

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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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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.

2.7. Quantitative PCR (qPCR)

Wastewater samples were quantified in duplicate by qPCR using the ABI 7500 Real-Time PCR System (Applied Biosystems, CA, USA). qPCR was performed as described by Fumian et al. (2010) using primers designed by Zeng et al. (2008). A standard curve (10^6–10^1 copies per reaction) was generated using tenfold serial dilutions of pTOPO vectors (Invitrogen, USA) containing the NSP3 target region. The qPCR reaction was performed in a final volume of 25 µl by using Environmental PCR Master Mix (Applied Biosystems, CA, USA). Amplification data was collected and analyzed using the Sequence Detection Software version 1.0 (Applied Biosystems, CA, USA). A test result was considered positive if a sigmoidal amplification curve crossed the threshold before 40 cycles and all positive and negative control reactions gave expected results. In order to establish the amplification efficiency and the limit of detection of the real-time PCR assays, ten-fold dilutions (10^6–10 copies) of the plasmid NSP3 standard were tested by duplicate as described above. Assay efficiency ($10^{(-1/\text{slope})}$) was calculated from the slope of the standard curve which was generated by plotting the log copy number versus the cycle threshold (Ct) value.

2.8. Statistical analysis

Wilcoxon and Kruskal–Wallis tests were applied for the viral load analysis. *P*-values lower than 0.05 indicated significant differences for the variables measured. The underlying structure of the sewage specimens and the meteorological variables dataset were explored using non-parametric by Spearman's rank test. To study the relationship between the rotavirus load in urban raw sewage and the temperature, the median (50th percentile) of the mean temperature (18 °C) from the years 2009–2011 was determined and the viral load by month was categorized into two groups: the first one included viral loads detected at mean temperature lower than or equal to the median temperature, and the second group included viral loads detected at mean temperature higher than the median temperature. The group differences were analyzed by Wilcoxon test. Also, rotavirus genotypes detected were categorized according to the median (50th percentile) of the mean temperature and analyzed by *Z* test for independent proportion. Statistical analyses were performed with InfoStat (Di

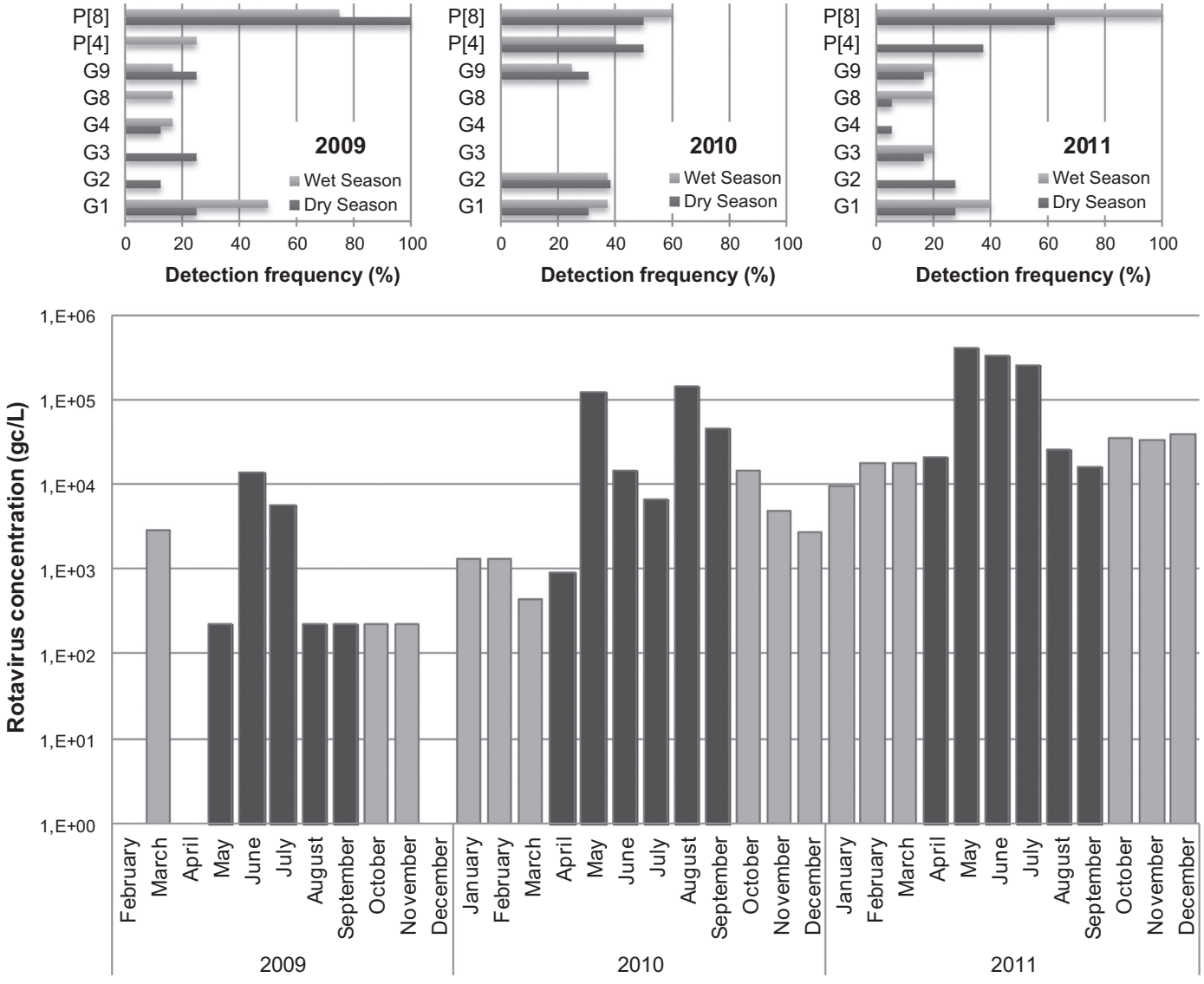

Fig. 1. Monthly variations in RV concentrations (genome copies/liter, gc/L) in raw sewage samples collected from Córdoba city, Argentina, during a 3-year period. The dry season (from April to September) is depicted with black bars, while the wet season (from October to March) is shown in gray. Seasonal RV genotypes prevalence rates are displayed above in bar graphs. The RV concentrations depicted as 0 (zero) were lower than 5 copies of target DNA per qPCR reaction.

Rienzo et al., 2014).

3. Results

3.1. qPCR sensitivity and detection limits

To validate the real-time PCR assay prior to the application to environmental samples, the detection limit and amplification efficiency were determined. Standard curves with 10-fold serial dilutions of plasmid NSP3 control (from 10^6 to 10 copies) were prepared and assayed in duplicate. The resulting standard curves, with strong correlation coefficients ($R^2 \geq 0.978$), indicated strong linear relationships. PCR amplification efficiency was calculated from the slope of the standard curves and was $\geq 99.85\%$. A detection limit of 10 copies of target DNA per reaction was determined, indicating high assay sensitivity.

3.2. Quantitative detection of rotavirus

Rotavirus concentration in local wastewater is depicted in Fig. 1.

The rotavirus genome was detected in 32 out of 35 sewage samples (91.4%) by qPCR and virus concentrations ranged from 2.2×10^2 to 4.1×10^5 genome copies/liter (gc/L) (mean 5×10^4 gc/L; CI 95% 2×10^4–8.8×10^4 gc/L). The statistical analysis showed significant different wastewater viral concentrations among the years studied (Kruskal Wallis test, $P=0.0003$). However, the three years displayed a similar annual pattern of rotavirus load in wastewater, being (for each of the three studied years) higher but not statistically significant between April and September (autumn and winter seasons, corresponding to the DS) as compared to the viral load detected between October and March (spring and summer, corresponding to the WS) (year 2009: April–September vs. October–March $P=0.71$; year 2010: April–September vs. October to March $P=0.07$; year 2011: April to September vs. October to March $P=0.31$, Wilcoxon test).

According to the results cited above, the dataset from meteorological variables and rotavirus concentration from each year studied (2009, 2010 and 2011) was divided in two seasonal groups based on the records of rainfall for the period studied. The DS spanned from April to September with 95.2 mm rainfall (mean rainfall in the 3-year study period), while the WS included the period from October to March with 538.5 mm rainfall (mean rainfall in the 3-year study period), matching the months of DS and WS with those in which different, but not statistically significant, rotavirus concentrations in wastewater were detected in the three years studied. The mean values of the meteorological parameters studied with confidence intervals (95%) and rotavirus load with the reported ranges by DS and WS according to the year studied (2009, 2010 and 2011) are summarized in Table 1.

There were no significant differences in the maximum, minimum and mean temperatures, neither in relative humidity by DS nor by WS, among the years studied ($P > 0.05$). These results revealed a steady profile of temperatures, cumulative precipitation, and relative humidity as well as rotavirus concentration pattern in raw sewage in the three-year period studied.

In order to explain the correlation among the variables: rotavirus load in wastewater samples vs. the maximum, minimum and mean temperatures, relative humidity and cumulative precipitation, a Spearman's rank analysis was performed on a standardized data set. The correlation among the variables was not significant; however, a trend of anti-correlation was noted for rotavirus concentration and temperature (mean temperature: $r=-0.40$, $P=0.0179$; minimum temperature: $r=-0.38$, $P=0.0227$; maximum temperature: $r=-0.40$, $P=0.0170$) and also with cumulative precipitation ($r=-0.25$, $P=0.1530$). Relative humidity ($r=-0.02$, $P=0.9311$) did not show a relationship with the rotavirus concentration (Fig. 2).

A significant higher rotavirus concentration in urban sewage was observed when the mean temperature was lower than or equal to 18 °C ($P=0.0056$), rendering an increase in rotavirus concentration in urban sewage of 8% (from 1.4×10^4 gc/L to 1.1×10^5 gc/L) (Fig. 2).

3.3. Molecular characterization of rotavirus strains

VP7-gene was successfully characterized in 29 out of the 35 sewage samples collected. Many sewage samples showed multiple genotypes in one sample as a mixture of viruses in sewage. During the 3-year period, all the common G genotypes were detected in the sewage, being the G1 (32.2%), G2 (23.7%) and G9 (22%) the dominant ones (Fig. 1). VP4-gene genotypes were characterized in a total of 22 (62.8%) sewage samples. The most common P genotype all over the study period and also in each particular year was the P[8] (67.9%), followed by P[4] (32.1%) (Fig. 1).

In order to analyze the impact of the environmental temperature on the distribution pattern of VP7 and VP4-genotypes detected in the urban raw sewage, the rotavirus G and P-genotypes that were detected monthly during the period 2009–2011 were categorized according to the median (50th percentile) of the mean temperature (18 °C). The results showed that the distribution of VP7 and VP4-genotypes detected at environmental temperatures equal to or lower than 18 °C was similar to the genotype distribution observed at temperatures higher than 18 °C. During the years 2009–2011, G-genotype frequencies at temperatures lower

Table 1
Mean values and range of confidence intervals (95%) of the meteorological parameters studied and rotavirus concentration according to the dry season (from April to September) and the wet season (from October to March) for the period 2009–2011.

Variable	2009		2010		2011	
	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season
Temperature (°C)	16.1	22.5	13.0	23.2	16.5	24.0
(95% CI)	(10.2–22.0)	(20.7–24.2)	(7.4–18.1)	(20.2–26.2)	(14.0–19-0)	(20.7–27.4)
Maximum temperature (°C)	22.3	30.5	20.2	30.5	24.5	31.1
(95% CI)	(18.6–26.0)	(27.9–31.1)	(15.0–25.5)	(27.5–33.5)	(22.0–26.9)	(27.1–35.2)
Minimum temperature (°C)	6.1	14	5.5	16	8.5	16.9
(95% CI)	(0.7–11.5)	(8.2–17.6)	(-0.4–11.4)	(11.7–20.3)	(5.8–11.3)	(14.1–19.7)
Relative humidity (%)	59.2	55.2	65.8	64.2	63.9	64.5
(95% CI)	(48.7–69.7)	(27.6–83.0)	(58.8–73.0)	(52.6–76.0)	(53.1–74.7)	(58.6–70.3)
Cumulative precipitation (mm)	48.8	528.0	176.2	534.1	60.6	553.3
Rotavirus concentration (10^3 gc/L)	4.0	1.1	57.3	4.2	176.6	25.6
(minimum–maximum values)	(0.2–13.7)	(0.2–2.9)	(0.90–148.8)	(0.4–14.8)	(16.1–405.0)	(9.4–39.5)

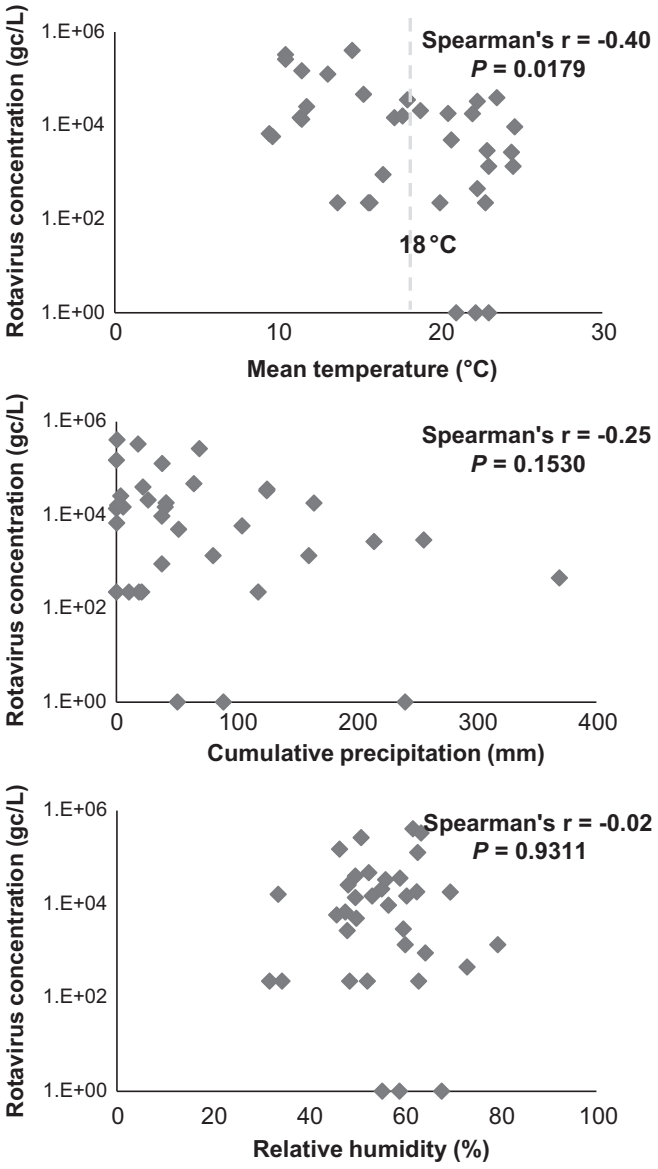

Fig. 2. Scatter plots of rotavirus concentration with meteorological factors (mean temperature, cumulative precipitation and relative humidity) from 2009 to 2011 in Córdoba, Argentina. Correlation was evaluated using non-parametric Spearman's rank test. The median (50th percentile) of the mean temperature (18 °C) is shown as a vertical dashed gray line. RV concentrations depicted as 0 (zero) were lower than 5 copies of target DNA per qPCR reaction.

than or equal to 18 °C were: G1 27.8%, G2 27.8%, G3 13.9%, G4 5.5% and G9 25%, and P-genotype frequencies were: P[4] 33.3% and P[8] 66.7%; meanwhile, at temperatures higher than 18 °C the following detection rates were observed: G1 47.4%, G2 21%, G3 10.5%, G4 5.3%, G8 5.3%, G9 10.5%, P[4] 30.8% and P[8] 69.2% (Z test for independent proportion >0.05).

4. Discussion

Rotavirus replication within the intestinal tract can result in the shedding of more than 10^{10} infectious particles per gram of feces that are excreted by both symptomatic and asymptomatic individuals. Thereby, the content of these viruses in sewage mirrors the infectious status of the population (Myrmel et al., 2006). Thus, environmental sampling of raw wastewater is a suitable approach for the study of circulating enteric viruses in a given population.

A quantitative PCR was employed in the present study in order to determine the rotavirus load in sewage water from Córdoba city. Also, conventional RT-nested PCR was applied for VP7 and VP4 viral genes characterization. Both techniques showed a relatively similar sensitivity in the rotavirus detection rate (32/35 rotavirus positive samples by qPCR and 29/35 by conventional nested PCR). Similar results in terms of rotavirus detection sensitivity were observed in wastewater samples from Rio de Janeiro, Brazil, where no significant statistical differences were found between conventional PCR and qPCR (Prado et al., 2011).

Monthly monitoring of Córdoba wastewater inlet by qualitative and quantitative PCR revealed the presence of rotavirus in cold and warm seasons, indicating its continuous circulation in the local community, and therefore suggesting that transmission occurs all year round. This issue raises the question about the dissimilar rotavirus disease incidence in Argentina during warm and cold weather. Probably, the asymptomatic infected individuals play an important role in the maintenance of the virus circulation in the summer time.

Higher rotavirus concentrations, although not statistically different, were seen in the DS (April–September) as compared with the WS (October–March). This seasonal pattern of rotavirus concentration in urban sewage was repeatedly observed during the 3-year study period, indicating that our study captured a unique and stable phenomenon that suggests a potential effect of local weather on the seasonal pattern of rotavirus circulation.

In order to increase the understanding of the rotavirus concentration pattern in local urban sewage, we assembled a local set of time series from the three consecutive study years and examined the relationship between the rotavirus concentration in raw wastewater and the meteorological factors by Spearman's correlation analysis. The correlation among the variables was not significant; however, a trend of negative correlation was noted between rotavirus concentration in sewage and temperature as well as with cumulative precipitation. The more detailed analysis revealed that the charge of rotaviruses in Córdoba sewage was significantly higher at mean temperatures lower than 18 °C and also higher, but not statistically different during DS. No association between rotavirus concentration and relative humidity was noted.

The present study also provided information about rotavirus VP7 and VP4 genes characterization in raw sewage. During the 3-year study period, genotypes G1 and P[8] were dominant. This result is in agreement with the epidemiology pattern of rotavirus that has been observed in the local community since the year 1979 in children with rotavirus gastroenteritis (Barril et al., 2006, 2013), and also in municipal sewage during the year 2006 (Barril et al., 2010). The VP7 and VP4 genotypes were distributed homogeneously in the two categories of mean temperature that showed to have an effect on rotavirus concentration (below 18 °C and equal to or higher than 18 °C). This result is in disagreement with other investigations were variable seasonal patterns of rotavirus genotypes were reported, showing an association of some genotypes with warm and rainy seasons and others with cold and dry seasons (Rahman et al., 2007; Enweronu-Laryea et al., 2013). Therefore, it is likely that seasonal patterns cannot be reinforced by changes in rotavirus genotypes.

Overall, our study shows that colder and drier weather has a direct effect on the circulation of rotavirus, which is shown as an increase of the viral load in urban wastewater. The dominance of rotavirus during these conditions resembles that of viral infections spread by the respiratory route, such as influenza and measles (Cook et al., 1990; Haffejee, 1995).

In nosocomial outbreaks of rotavirus gastroenteritis, many patients show symptoms of upper respiratory tract infection before the onset of diarrhea, although the rotavirus has not been isolated from the respiratory tract (Ansari et al., 1991). Bishop proposes

that the explanation might lie in the airborne spread of aerosolized particles that are ingested, rather than through respiratory tract infections (Bishop, 1996). In this way, the airborne component of rotavirus transmission might have an important role in rotavirus seasonality. However, it is important to acknowledge the limitations of this research. The abundance of rotavirus may fluctuate daily in raw sewage and the samples analyzed in this study represent only a single point in time. Anyway, all samples were collected at the same time range throughout the study period in order to minimize the effects of diurnal variations. Another limitation of the study is that the results may have been biased by artificial virus concentration in drier months, due to lower rainfall during this season. Meanwhile, during the WS, the same virus amount might be diluted by higher rainfall. To minimize this issue, sewage samples were not collected on rainy days or after a rainy day.

The circulation of rotavirus all year round capture a phenomenon of rotavirus spread that is complex and it is unlikely that weather conditions alone account directly for the broad seasonality of rotavirus disease in winter. We speculate that the stability and survivability of the virus, alternative transmission routes, the virus/host interaction, and also temporal changes in human susceptibility and behavior, including sanitation and hygiene practices, and probability of environmental exposures, might all together be involved in the seasonal pattern of rotavirus disease.

5. Conclusions

Rotavirus circulates in the local community all year round, which suggests that the transmission occurs throughout the year. However, a slight trend for rotavirus circulation is noted during the colder and drier months of the year. Higher rotavirus concentrations were associated with a decline in the environmental mean temperature (especially lower than 18 °C, $P=0.0056$) and cumulative precipitation (although not statistically significant) but were not related with variations in the ambient relative humidity. During the period 2009–2011, rotavirus genotypes G1, G2, G9, P[4] and P[8] were frequently detected in the urban sewage of Córdoba, Argentina. No association between the VP7 and VP4 genotypes and the meteorological variables was observed. The results obtained suggest that drawing the attention to local climatic conditions, which in term impact directly in human behavior, will improve our understanding of the transmission and epidemiology of the rotavirus disease.

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References

Ansari, S., Springthorpe, S., Sattar, S., 1991. Survival and vehicular spread of human rotaviruses: possible relation to seasonality of outbreaks. Rev. Infect. Dis. 13, 448–461.
Atchison, C.J., Tam, C.C., Lopman, B.A., 2009. Season of birth and risk of rotavirus diarrhoea in children aged < 5 years. Epidemiol. Infect. 137, 957–960.
Barril, P.A., Martinez, L.C., Giordano, M.O., Castello, A.A., Rota, R.P., Isa, M.B., Masachessi, G., Ferreyra, L.J., Glikmann, G., Nates, S.V., 2006. Detection of group a human rotavirus G9 genotype circulating in Córdoba, Argentina, as early as 1980. J. Med. Virol. 78, 1113–1118.
Barril, P.A., Giordano, M.O., Isa, M.B., Masachessi, G., Ferreyra, L.J., Castello, A.A., Glikmann, G., Nates, S.V., 2010. Correlation between rotavirus A genotypes detected in hospitalized children and sewage samples in 2006, Córdoba, Argentina. J. Med. Virol. 82, 1277–1281.
Barril, P., Martínez, L., Giordano, M., Masachessi, G., Isa, M., Pavan, J., Glikmann, G., Nates, S., 2013. Genetic and antigenic evolution profiles of G1 rotaviruses in Córdoba, Argentina, during a 27-year period (1980–2006). J. Med. Virol. 85, 363–369.
Bishop, R.F., 1996. Natural history of human rotavirus infection. Arch. Virol. Suppl. 12, 119–128.
Bok, K., Castagnaro, N., Borsa, A., Nates, S., Espul, C., Fay, O., Fabri, A., Grinstein, S., Miceli, I., Matson, D., Gomez, J., 2001. Surveillance for rotavirus in Argentina. J. Med. Virol. 65, 190–198.
Cook, S.M., Glass, R.I., LeBaron, C.W., Ho, M.S., 1990. Global seasonality of rotavirus infections. Bull. World Health Organ. 68, 171–177.
Dennehy, P.H., 2008. Rotavirus vaccines: an overview. Clin. Microbiol. Rev. 21, 198–208.
Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C. W., 2014. InfoStat Version 2014. Grupo InfoStat, FCA. Universidad Nacional de Córdoba, Argentina.
D'Souza, R.M., Hall, G., Becker, N.G., 2008. Climatic factors associated with hospitalizations for rotavirus diarrhoea in children under 5 years of age. Epidemiol. Infect. 136, 56–64.
Enweronu-Laryea, C.C., Sagoe, K.W., Damanka, S., Lartey, B., Armah, G.E., 2013. Rotavirus genotypes associated with childhood severe acute diarrhoea in southern Ghana: a cross-sectional study. Virol. J. 10, 287.
Estes, M.K., 2001. Rotaviruses and their replication In: Knipe, D.M., Howley, R.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B., Straus, S.E. (Eds.), Fields Virology, 4th ed. Lippincott Williams & Wilkins, Philadelphia, pp. 1747–1785.
Fumian, T.M., Leite, J.P., Castello, A.A., Gaggero, A., Caillou, M.S., Miagostovich, M.P., 2010. Detection of rotavirus A in sewage samples using multiplex qPCR and an evaluation of the ultracentrifugation and adsorption–elution methods for virus concentration. J. Virol. Methods 170, 42–46.
Gentsch, J.R., Glass, R.I., Woods, P.A., Gouvea, V., Gorziglia, M., Flores, J., Das, B.K., Bhan, M.,K., 1992. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J. Clin. Microbiol. 30, 1365–1373.
Gouvea, V., Glass, R.I., Woods, P., Taniguchi, K., Clark, H.F., Forrester, B., Fang, Z.Y., 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J. Clin. Microbiol. 28, 276–282.
Greening, G.E., Hewitt, J., Lewis, G.D., 2002. Evaluation of integrated cell 3 culture-PCR (C-PCR) for virological analysis of environmental samples. J. Appl. Microbiol. 93, 745–750.
Haffejee, I.E., 1995. The epidemiology of rotavirus infections: a global perspective. J. Pediatr. Gastroenterol. Nutr. 20, 275–286.
Herring, A.J., Inglis, N.F., Ojeh, C.K., Snodgrass, D.R., Menzies, J.D., 1982. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid silver–stain polyacrilamide gels. J. Clin. Microbiol. 16, 473–477.
Huang, Q.S., Greening, G., Baker, M.G., Grimwood, K., Hewitt, J., Hulston, D., van Duin, L., Fitzsimons, A., Garrett, N., Graham, D., Lennon, D., Shimizu, H., Miyamura, T., Pallansch, M.A., 2005. Persistence of oral polio vaccine virus after its removal from the immunization schedule in New Zealand. Lancet 366, 394–396.
Iturriza-Gomara, M., Green, J., Brown, D.W., Desselberger, U., Gray, J.J., 2000. Diversity within the VP4 gene of rotavirus P[8] strains: implications for reverse transcription-PCR genotyping. J. Clin. Microbiol. 38, 898–901.
Jagai, J.S., Sarkar, R., Castronovo, D., Kattula, D., McEntee, J., Ward, H., Kang, G., Naumova, E.N., 2012. Seasonality of rotavirus in South Asia: a meta-analysis approach assessing associations with temperature, precipitation, and vegetation index. PLoS One 7, e38168.
Kapikian, A.Z., Hoshino, Y., Chanock, R.M., 2001. Rotaviruses In: Knipe, D.M., Howley, R.M. (Eds.), Fields Virology, 4th ed. Lippincott Williams & Wilkins, Philadelphia, pp. 1787–1883.
Kargar, M., Sadeghipour, S., Nategh, R., 2009. Environmental surveillance of non-polio enteroviruses in Iran. Virol. J. 6, 149.
Levy, K., Hubbard, A.E., Eisenberg, J.N., 2009. Seasonality of rotavirus disease in the tropics: a systematic review and meta-analysis. Int. J. Epidemiol. 38, 1487–1496.
Lewis, G.D., Metcalf, T.G., 1988. Polyethylene glycol precipitation for recovery of pathogenic viruses, including hepatitis A virus and human rotavirus, from oyster, water, and sediment samples. Appl. Environ. Microbiol. 54, 1983–1988.
Meleg, E., Bányai, K., Martella, V., Jiang, B., Kocsis, B., Kisfali, P., Melegh, B., Szucs, G., 2008. Detection and quantification of group C rotaviruses in communal sewage. Appl. Environ. Microbiol. 74, 3394–3399.
Mueller, J.E., Bessaud, M., Huang, Q.S., Martinez, L.C., Barril, P.A., Morel, V., Balanant, J., Bocacao, J., Hewitt, J., Gessner, B.D., Delpeyroux, F., Nates, S.V., 2009. Environmental poliovirus surveillance during oral poliovirus vaccine and inactivated poliovirus vaccine use in Córdoba Province. Argentina, Appl. Environ. Microbiol. 75, 1395–1401.
Myrmel, M., Berg, E.M., Grinde, B., Rimstad, E., 2006. Enteric viruses in inlet and outlet samples from sewage treatment plants. J. Water Health 4, 197–209.
Parashar, U.D., Burton, A., Lanata, C., Boschi-Pinto, C., Shibuya, K., Steele, D., Birmingham, M., Glass, R.I., 2009. Global mortality associated with rotavirus disease among children in 2004. J. Infect. Dis. 13, S9–S15.
Pitzer, V.E., Viboud, C., Simonsen, L., Steiner, C., Panozzo, C.A., Alonso, W.J., Miller,

M.A., Glass, R.I., Glasser, J.W., Parashar, U.D., Grenfell, B.T., 2009. Demographic variability, vaccination, and the spatiotemporal dynamics of rotavirus epidemics. Science 325, 290–294.
Pitzer, V.E., Viboud, C., Lopman, B.A., Patel, M.M., Parashar, U.D., Grenfell, B.T., 2011. Influence of birth rates and transmission rates on the global seasonality of rotavirus incidence. J. R. Soc. Interface 8, 1584–1593.
Prado, T., Silva, D.M., Guilayn, W.C., Rose, T.L., Gaspar, A.M., Miagostovich, M.P., 2011. Quantification and molecular characterization of enteric viruses detected in effluents from two hospital wastewater treatment plants. Water Res. 45, 1287–1297.
Rahman, M., Sultana, R., Ahmed, G., Nahar, S., Hassan, Z.M., Saiada, F., Podder, G., Faruque, A.S., Siddique, A.K., Sack, D.A., Matthijnssens, J., Van Ranst, M., Azim, T., 2007. Prevalence of G2P[4] and G12P[6] rotavirus, Bangladesh. Emerg. Infect. Dis. 10, 18–24.
Ruiz-Palacios, G.M., Pérez-Schael, I., Velázquez, F.R., Abate, H., Breuer, T., Clemens, S. C., Cheuvart, B., Espinoza, F., Gillard, P., Innis, B.L., Cervantes, Y., Linhares, A.C., López, P., Macías-Parra, M., Ortega-Barría, E., Richardson, V., Rivera-Medina, D. M., Rivera, L., Salinas, B., Pavía-Ruz, N., Salmerón, J., Rüttimann, R., Tinoco, J.C., Rubio, P., Nuñez, E., Guerrero, M.L., Yarzábal, J.P., Damaso, S., Tornieporth, N., Sáez-Llorens, X., Vergara, R.F., Vesikari, T., Bouckenooghe, A., Clemens, R., De Vos, B., O'Ryan, M., 2006. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N. Engl. J. Med. 354, 11–22.
Ursu, K., Kisfali, P., Rigó, D., Ivanics, E., Erdélyi, K., Dán, A., Melegh, B., Martella, V., Bányai, K., 2009. Molecular analysis of the VP7 gene of pheasant rotaviruses identifies a new genotype, designated G23. Arch. Virol. 154, 1365–1369.
Vesikari, T., Matson, D.O., Dennehy, P., Van Damme, P., Santosham, M., Rodriguez, Z., Dallas, M.J., Heyse, J.F., Goveia, M.G., Black, S.B., Shinefield, H.R., Christie, C.D., Ylitalo, S., Itzler, R.F., Coia, M.L., Onorato,, M.T., Adeyi, B.A., Marshall, G.S., Gothefors, L., Campens, D., Karvonen, A., Watt, J.P., O'Brien,, K.L., DiNubile, M.J., Clark, H.F., Boslego, J.W., Offit, P.A., Heaton, P.M., 2006. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. N. Engl. J. Med. 354, 23–33.
Zeng, S.Q., Halkosalo, A., Salminen, M., Szakal, E.D., Puustinen, L., Vesikari, T., 2008. One-step quantitative RT-PCR for the detection of rotavirus in acute gastroenteritis. J. Virol. Methods 153, 238–240.