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Rotavirus Monitoring in Drinking and Surface Waters of Cordoba, Argentina

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ABSTRACT

Enteric viruses are the major causative agents of waterborne diseases, however there are no regulations that control their presence in aqueous matrices. It has been proved that current standards for microbiological water quality do not guarantee the absence of viruses. The aims of this study were to analyze the presence and concentration of rotavirus, the main etiological agent of infantile gastroenteritis, in environmental waters of Córdoba, Argentina, and to estimate the risk of waterborne rotavirus infection. A total of 101 samples were analyzed (25 drinking waters and 76 surface waters from the San Roque Dam, Suquía River and Xanaes River). Two different methodologies for virus concentration were applied, filtration with negative charged membranes followed by ultracentrifugation for drinking waters and polyethylene glycol precipitation for surface waters. Rotavirus detection and quantification was assayed by RT-real time PCR. Quantitative microbiological risk assessment **(QMRA)** of the environmental waters was undertaken for rotavirus by β-Poisson modeling and Monte Carlo simulation. The drinking waters did not reveal rotavirus contamination, while a total of 37/76 (48.7%) surface waters showed rotavirus presence. The San Roque Dam and Suquía River revealed rotavirus presence in all the samples (28/28, 100%) at high concentrations ($1.1x10^6$ gc/L and $5.2x10^5$gc/L, respectively). The Xanaes River exhibited a lower level of viral contamination, being rotavirus sporadically detected (9/48, 18.7%) at an average concentration of $8.5x10^0$ gc/L. QMRA revealed a risk of rotavirus infection of 0.18 and 0.77 for an individual exposure to the Xanaes and Suquía Rivers and 0.85 by contacting with the San Roque Dam. Rotavirus monitoring should be included in water quality assessment of surface waters in order to alert the population about the health risks by contact with contaminated waters. In contrast, the consumption of drinking water in Córdoba city would not be a risk for transmission of rotavirus to the population exposed.

Keywords: Rotavirus; Environmental surveillance; Viral contamination; Water pollution; Quantitative microbiological risk assessment **(QMRA)**

INTRODUCTION

The continuous discharge of untreated or partially treated sewage into surface waters contributes directly to the introduction and circulation of viral pathogens in the aquatic environment [1]. However, despite the release of large quantities of human viruses into rivers, surface water is used widely for recreational activities, agricultural irrigation and also for the production of drinking water [2,3,4,5]. Taking into consideration the water usage for essential activities, this contamination scenario may pose major health risks. The role of water in the transmission of infectious diseases is well known. Nearly 25% of the global population (1.8 billion people in 2012) is consuming fecally-contaminated water [6]. Unsafe water, inadequate sanitation and poor hygiene are responsible for about 90% of diarrheal deaths worldwide [7].

Among the gastroenteric viruses, rotaviruses have emerged as an important agent of acute infantile gastroenteritis, being associated with approximately 453,000 deaths annually [8]. Rotaviruses are icosahedral particles consisting of 11 segments of double-stranded RNA surrounded by a triple capsid protein. The outer capsid proteins, VP7 and VP4, contain epitopes that induce neutralizing antibodies in the infected host and define specific G (VP7) and P (VP4) serotypes. Considering the burden of infections caused by rotavirus, two oral attenuated vaccines were licensed in the year 2006, one pentavalent vaccine, RotaTeq® (Merck Sharp & Dohme); and one monovalent vaccine, Rotarix® (GlaxoSmithKline). Rotarix® was introduced in the National Immunization Program of Argentina in January 2015 [9]. Rotavirus vaccination has shown to be effective in preventing hospitalization and severe rotavirus disease with varied efficacy and effectiveness by region, among children under five years old [10,11].

Microbiological indicators of water quality, such as *Escherichia coli* and *Enterococci*, are widely used in the monitoring programs for regulatory control [12-14]. These indicators may also point out the presence of other non-bacterial pathogens, such as enteric viruses and parasitic protozoa [15]. However, the absence of *Escherichia coli* and *Enterococci* does not exclude the presence of enteric viruses which are generally more resistant than bacteria to sewage treatment procedures

[16,17], and there is not regulation that determines the control of enteric viruses in aqueous matrices.

Quantitative microbial risk assessment **(QMRA)** is a growing and diversifying area of research for public health. QMRA seeks to evaluate the risk of adverse health effects, particularly infection, resulting for human exposures to infectious microbes [18]. It is a probabilistic modeling technique that is now widely used in assessing health risks associated with exposure to waterborne pathogens.

The aims of this study were to analyze the presence and viral load of rotavirus in drinking and surface waters of Córdoba, Argentina, and to estimate the risk of rotavirus infection by water consumption.

MATERIALS AND METHODS

Background

Córdoba city is the capital of the Province of Córdoba, located in the central region of Argentina and has approximately 1,317,298 inhabitants with a population density of 2,308 habitants/km^2. Suquía River rises in the San Roque Dam and traverses Córdoba city from west to east. Suquía water flow is 10 m^3/s, subject to a seasonal fluctuation: high flow during the WS (24 m^3/s) and very low during the DS (1.5 m^3/s). The San Roque Dam is located in a sierras system at the Punilla valley, beside the city of Carlos Paz, 40 km west of Córdoba city. San Roque Dam is a human-made reservoir constructed more than 70 years ago for flood control and water supply. The Dam has two main tributary rivers: the San Antonio River (estimated annual mean of 4 m^3/s) and the Cosquin River (estimated annual mean of 16.5 m^3/s) both rivers subject to a seasonal fluctuation in water flow. The surface area of this reservoir covers about 16 km^2 which attracts a good deal of tourists to the area. Fishing, swimming and sailing are some of the activities practiced in and around its waters, thus promoting an increase in the urbanization of the surroundings of the dam. Also, San Roque Dam is the source of water supply to approximately 70% of Córdoba city. The water is channelled from the San Roque Dam to the Suquía potable water treatment plant (located at km 10.5 of La Calera Road) and then the treated water is distributed to the city of Córdoba by culverts to be used as drinking water.

Villa del Rosario is the head town of Río Segundo Department (Province of Córdoba). It has 15,313 inhabitants and a population density of 86 habitants/km^2. It is located 80 km east-southeast from Córdoba city, on the right-hand banks of the Xanaes River, which is born in the Paravachasca Valley at the confluence of the Los Molinos and Anizacate Rivers, on the eastern slopes of the Cumbres de Achala. Xanaes River flows west-east with an average inflow of 12.2 m^3/s.

Monitoring Sites and Sample Collection

Surface water samples (n=76) were collected from two different monitoring areas of Córdoba province (Figure 1A). The first area, named Córdoba city and surrounded included one monitoring station in the San Roque Dam and seven monitoring stations over the Suquía River (Figure 1B). Three stations were located upstream from Córdoba city (1-San Roque Dam, a middle-dam monitoring site, 2-Villa Warcalde and 3-San Antonio ford), three throughout Córdoba city (4-Zipoli bridge, 5-Centenario bridge and 6-Sargento Cabral ford) and one located downstream from Córdoba city and the main sewage treatment plant of the city (7-San José bridge). The sampling was carried out twice during the dry season (DS, April-September, average temperature 13.6°C with 146.1 mm of rainfall) and also twice during the wet season (WS, October-March, average temperature 21.5°C with 723.8 mm of rainfall) of the year 2010, collecting a total of 28 surface water samples (4 from the San Roque Dam and 24 from the Suquía River). The second monitoring area, named Villa del Rosario included four monitoring stations over the Xanaes River (Figure 1C). One station was located upstream the city (1-Farm), two throughout (2-Coast and 3-Bridge) and one downstream (4-Stage). A monthly monitoring was done during a one-year period (December 2011-November 2012), collecting a total of 48 water samples (6 samples per monitoring station during the DS and 6 during the WS).

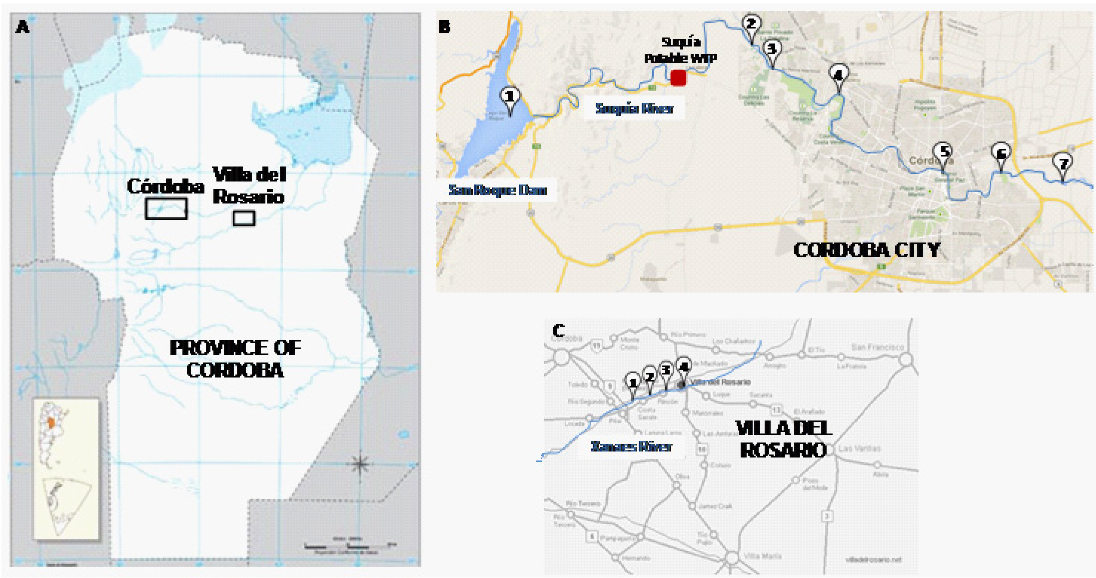

Figure 1: Sampling location sites in Córdoba bathing waters. A) Sampling geographic areas indicated in Córdoba Province (Argentina) map: Córdoba city and surrounding area and Villa del Rosario city. Thick lines in blue indicate the rivers and dam, numbers above them depict the monitoring stations: B) San Roque Dam and Suquía River: 1-San Roque Dam; 2-Villa Warcalde; 3-San Antonio ford; 4-Zipoli bridge; 5-Centenario bridge; 6-Sargento Cabral ford; 7-San José bridge; in red is shown the Suquía Potable Water Treatment Plant; C) Xanaes River: 1-Farm; 2-Coast 3-Bridge; 4-Stage.

Also, an exploratory study of the virological quality of treated drinking water of the city of Córdoba, Argentina, was performed. The origin source of the water collected was the water reservoir San Roque, which was subsequently potabilized at the Suquia water treatment plant. Water samples were collected between March 30 and April 10, 2015 from 25 different homes randomly selected, located in the center area of the city of Cordoba (i.e. in the City Center and New Córdoba neighborhoods). The water samples were collected directly from the tap.

Drinking and surface water samples were collected using sterilized plastic water storage containers (volume 2 L and 1.5 L respectively). Samples were transported within 12 hr at 4ºC to 8ºC to the laboratory, for further processing and analysis.

Virus Concentration

Viruses were concentrated from the drinking waters (4000X) using an adsorption-elution/filtration method based on negatively charged membranes [19] followed by an ultracentrifugation-based method. Surface water samples were concentrated (100 X) by the method of polyethylene glycol precipitation [20,21].

Nucleic Acid Extraction and cDNA Synthesis

Viral RNA was extracted using the commercial QIAamp Viral RNA kit (Qiagen Inc., Hilden, Germany). Extracted RNA was reverse-transcribed into cDNA using random hexamer primers and AMV reverse transcriptase.

Rotavirus Detection and Quantification

Water samples were detected and quantified in duplicate by qPCR using the ABI 7500 Real-Time PCR System (Applied Biosystems, CA, USA). qPCR was performed as described by [22] using primers designed by [23]. A standard curve (10^6 to 10^1 copies per reaction) was generated using tenfold serial dilutions of pTOPO vectors (Invitrogen, CA, USA) containing the NSP3 target region. Positive and negative controls were included. Assay efficiency ($10^{(-1/slope)}$) was calculated from the slope of the standard curve which was generated by plotting the log copy number versus the cycle threshold **(Ct)** value. The recovery efficiencies of the nucleic acid extraction and concentration procedures were considered for the determination of rotavirus concentration in the initial waters [24].

Quantitative Microbial Risk Assessment

The β- Poisson dose-response model was used to estimate the probability of rotavirus infection [25]: α = 0.2531 and N50 = 6.17). The following underlying assumptions were used in the exposure assessment for rotavirus: i) exposure through direct contact by playing and swimming in the surface waters; ii) involuntary ingestion of 10 mL/exposure of surface water, and the annual volume of involuntary water ingested was based on a frequency of 6 events per year for surface water [26-29]; iii) the ratio of infectious virus particles to total detected virus particles was 1:10 [30,31]. The uncertainty was introduced through the analysis of data distribution by sampling points. Monte Carlo simulations were made for 10,000 iterations using @Risk software 6.3 (Palisade Corporation, Newfield, New York). In each iteration, samples were taken from the data distribution function. The output of the analysis was the mean and standard error of the risk of infection as well as the frequency distribution of the probabilities of infection. The recalculated values were plotted in a box and whisker plot in order to show the extreme values and the range of middle values.

RESULTS

The drinking waters analyzed did not show rotavirus presence (0/25; 0%) after concentrating the viral particles 4000 X using an adsorption-elution method with negatively charged membranes followed by ultracentrifugation. The detection of viral genomes in treated water of different regions is not an usual event [32]; however some studies have reported the presence of enteric viruses in tap water treated by conventional processes [33-35]. In our study it is noteworthy that the source of drinking water is the water of San Roque Dam, where high concentration of rotavirus was detected all around the year. Although in this study the monitoring of the surface and drinking waters was carried out during different periods, a more extensive monitoring of the San Roque Dam waters covering the years 2012-2015 also revealed high rotavirus concentrations all over the period (data not shown). Therefore, not detecting rotavirus in the drinking waters suggests that the methodology used in the Suquia potable water treatment plant is efficient for the removal of viruses. Anyway, it would be interesting to extend and carry out a sustained sampling of drinking water in order to monitor the presence of viruses in water.

A total of 37/76 (48.7%) surface waters revealed rotavirus presence. All the water samples collected from the San Roque Dam and Suquía River showed rotavirus presence, always at high concentrations. The Xanaes River exhibited a lower level of viral contamination, being rotavirus sporadically detected (18.7%), but always at high concentrations (Table 1). The high rate of rotavirus detection in the recreational waters of the San Roque Dam and Suquía River is consistent with the prevalence of enteric viruses in various aqueous matrices of the region and the world which present large urban settlements on its banks [36,37]. On the other hand, the lower risk of rotavirus infection in the Xanaes River is in line with reports of viral quality of surface waters less impacted by human activities [38,39].

Table 1: Rotavirus detection and concentration in drinking and surface waters of Córdoba, Argentina.

Collection areas	Number of samples	Rotavirus		
		Presence n (%)	Mean concentration (gc/L) (range)	SD†
Drinking water	25	0 (0)	NA‡	NA
San Roque Dam	4	4 (100)	$2.1x10^6$ ($2.0x10^5$-$6.1x10^6$)	$2.7x10^6$
Suquía River	24	24 (100)	$2.7x10^6$ ($1.9x10^3$-$8.6x10^6$)	$2.7x10^6$
Xanaes River	48	9 (18.7)	$8.5x10^0$ (0-$3x10^6$)	$4.6x10^5$

†**SD**: Standard deviation. ‡**NA**: Not applied.

The deterioration in the viral quality when passing through the cities was evaluated for the environmental waters monitored (Figure 2). Viral loads obtained from the San Roque middle-dam monitoring site were compared to those of the Suquía River, as this dam gives rise to the Suquía River. The San Roque/Suquía complex revealed the highest rotavirus concentration in the monitoring site 6-Sargento Cabral ford and the lowest viral concentrations in 3-San Antonio ford and 4-Zipoli Bridge; however the differences with viral concentrations from other monitoring stations were not statistically significant ($P\geq0.20$). The Xanaes River revealed the highest rotavirus concentration in the monitoring site 1-Farm, while the station 3-Bridge revealed the lowest viral concentration, but these differences were not statistically significant ($P\geq0.23$). The similarity in rotavirus concentrations between the monitoring stations suggests that the selection of one point in the San Roque/Suquía complex and one in the Xanaes River would be representative of the whole monitoring area, thus reducing the number of sites that need to be analyzed in regular monitoring.

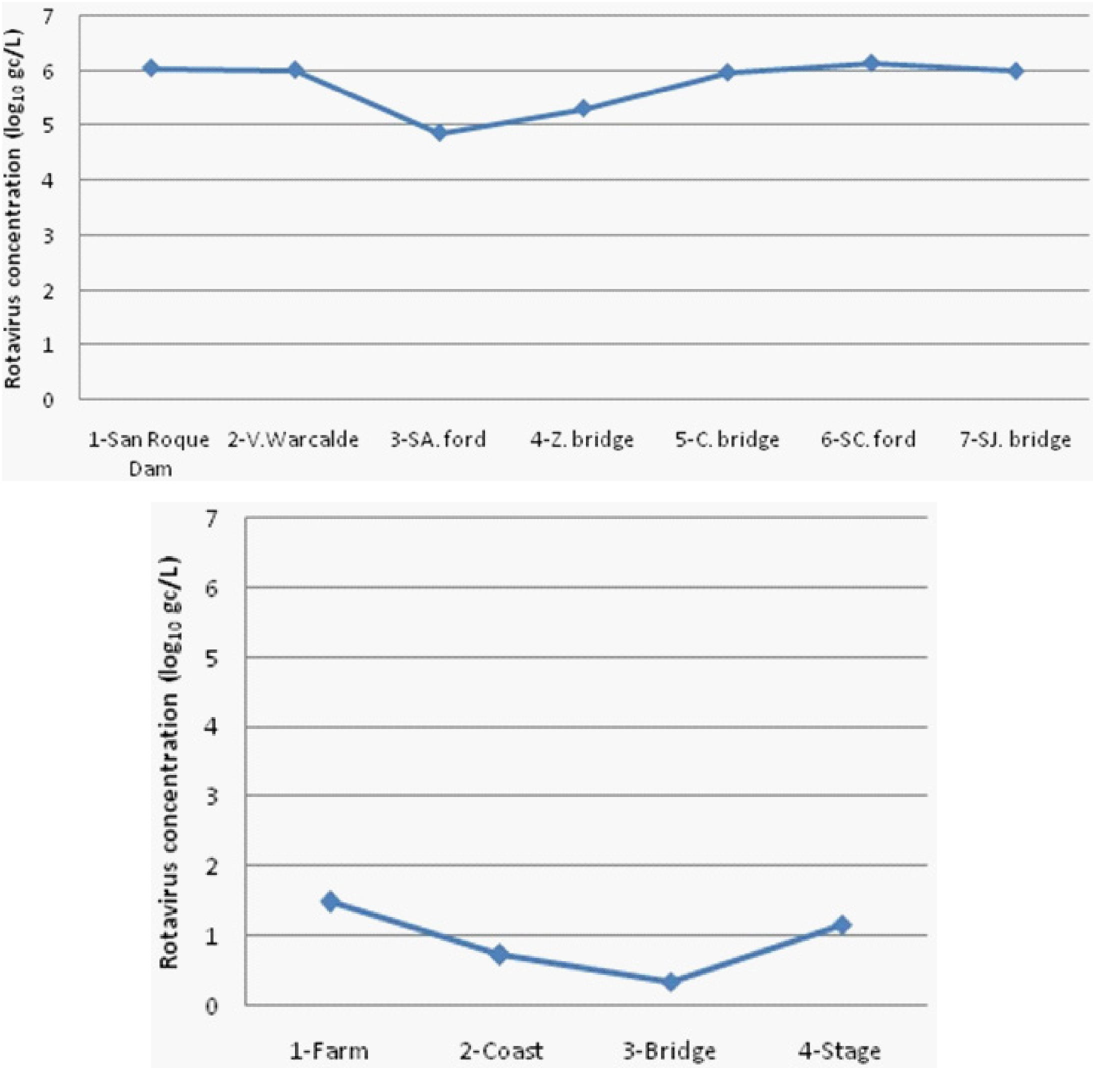

Figure 2: Rotavirus concentration at each sampling site of the (A) San Roque Dam/Suquía River complex and (B) Xanaes River.

The probability of infection from waterborne rotavirus was estimated for each of the exposure source points over the San Roque Dam, Suquía River and Xanaes River (Figure 3). The QMRA revealed a daily estimated risk of waterborne rotavirus infection associated with individual exposed to the Xanaes River of 0.18, meanwhile the daily risk by individual exposure to the San Roque Dam and Suquía River proved to be very high, 0.85 and 0.77 respectively (Figure 3). Annual health risk of rotavirus infection was high for the three monitoring water bodies (Xanaes: 0.7; Suquía 1 and San Roque 1), which reveals a public health hazard for the population exposed to these surface waters.

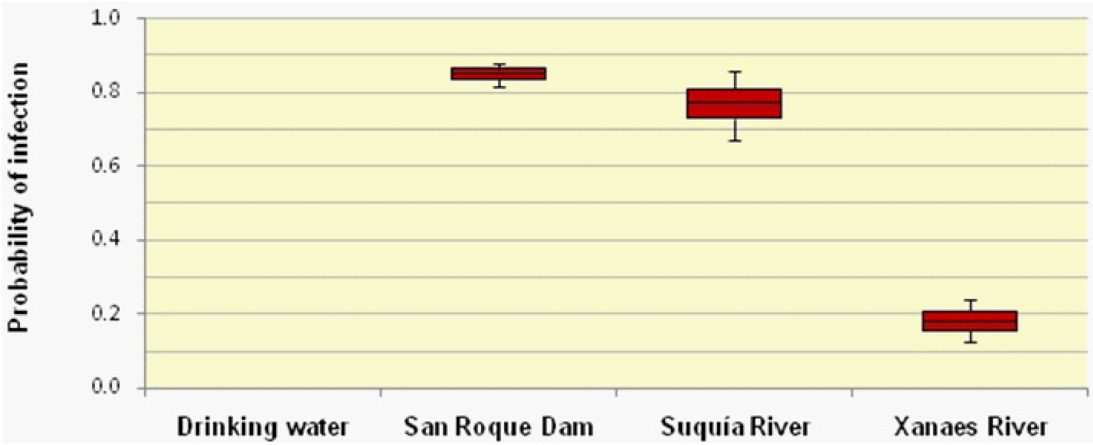

Figure 3: Daily estimated risk of waterborne rotavirus infection associated with individuals exposed to drinking and surface waters. The probability of infection is established in the 0–1 range. Boxplots represent 25th, 50th and 75th percentiles (bottom, middle and top edge of box) and outliers represent 5th and 95th percentiles.

The viral contamination detected in the recreational waters of Córdoba provides an advice of the probability of rotavirus infection for the population exposed to these polluted recreational waters. A combination of sanitation and hygiene intervention is required to minimize the risk of infection constituted by waterborne rotavirus in the identified sources of contamination. In addition this would help maintain the virological quality of drinking water.

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