

## Rotavirus contamination of surface waters from the northwest of Argentina

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### ABSTRACT

Fecal pollution of water is a serious concern because it is associated with the transmission of pathogens. The aim of this study was to analyze the occurrence of group A rotavirus (RVA) in surface waters from the Arias–Arenales River in Salta, a northern city in Argentina, and to define possible sources of fecal viral pollution. A total of 116 water samples were analyzed and RVA was detected in 3.4% (95% CI: 0.1–7.0%), with concentrations ranging from  $1.9 \times 10^5$  to  $3.8 \times 10^6$  genome copies per liter. RVA strains were characterized as G1P[8], G4P[8] and G9P[8], which are common genotypes circulating in the local population. The Arias–Arenales River presented unusual and sporadic contamination by RVA, originated from stormwater discharges and a variety of non-identified sources, and support the essential need of viral indicators for enhanced monitoring of water quality.

**Key words** | environmental surveillance, non-point contamination, point contamination, rotavirus, surface water, water quality

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## INTRODUCTION

Several kinds of enteric viruses causing water-related diseases with impact on public health are present in fecally contaminated water environments. Group A rotavirus (RVA), which is the most important causative factor of acute gastroenteritis in young children, is widespread worldwide, and its presence in different aquatic matrices is well documented (Prez *et al.* 2015; Elmahdy *et al.* 2016; Mackowiak *et al.* 2018). High viral shedding in feces of infected individuals, resistance to adverse environmental conditions and a low minimal infective dose make RVA an important environmental contaminant (Estes & Greenberg 2013).

Surface water bodies can be polluted by point-source discharges which enter into the environment from community sewage treatment plants, untreated sewage and livestock concentrations derived from slaughterhouse discharges (Jamieson *et al.* 2004; Collins *et al.* 2005). Also, non-point sources leading to diffuse pollution of surface waters may originate from direct fecal voiding by grazing livestock, manure spreading or urban surface water runoff, which can derive from roof and road surfaces contaminated with avian, domestic and wild animal feces and also from cross-connections to the urban sewerage system, all of which can exhibit high microbial loads (Sercu *et al.* 2011; Rusinol *et al.* 2014).

Since enteric viruses may be present at low concentrations in aqueous matrices, efficient concentration methods coupled with sensitive detection assays should be applied for viral recovery. Numerous studies have shown that ultrafiltration is a reliable and consistent method for sample concentration when applied to natural waters (Winona *et al.* 2001; Poma *et al.* 2012). Unlike other filtration systems, the recovery of viruses using ultrafiltration is largely unaffected by complex chemical constituents found in natural water (Morales-Morales *et al.* 2003). For viral detection, molecular methods allow the specific and fast detection of a target viral sequence, and in the case of real-time polymerase chain reaction (qPCR), it also allows the quantification of the target sequences. For the detection of viral pathogens, it is recommended the inclusion of an internal process control (IPC), like the bacteriophage PP7 of *Pseudomonas aeruginosa*, to determine whether the concentration

method or the presence of inhibitors affects viral recovery. PP7 has the particularity of its similarity in size and physico-chemical properties to poliovirus, the smallest member of the Enterovirus family. Consequently, PP7 simulates a worst-case scenario for the filtration of viruses, and also, there are no reports of its occurrence in natural waters (Rajal *et al.* 2007).

The present study aimed to assess RVA dissemination in the Arias–Arenales River, located in the province of Salta, northwest area of Argentina, and to identify possible sources of fecal viral pollution.

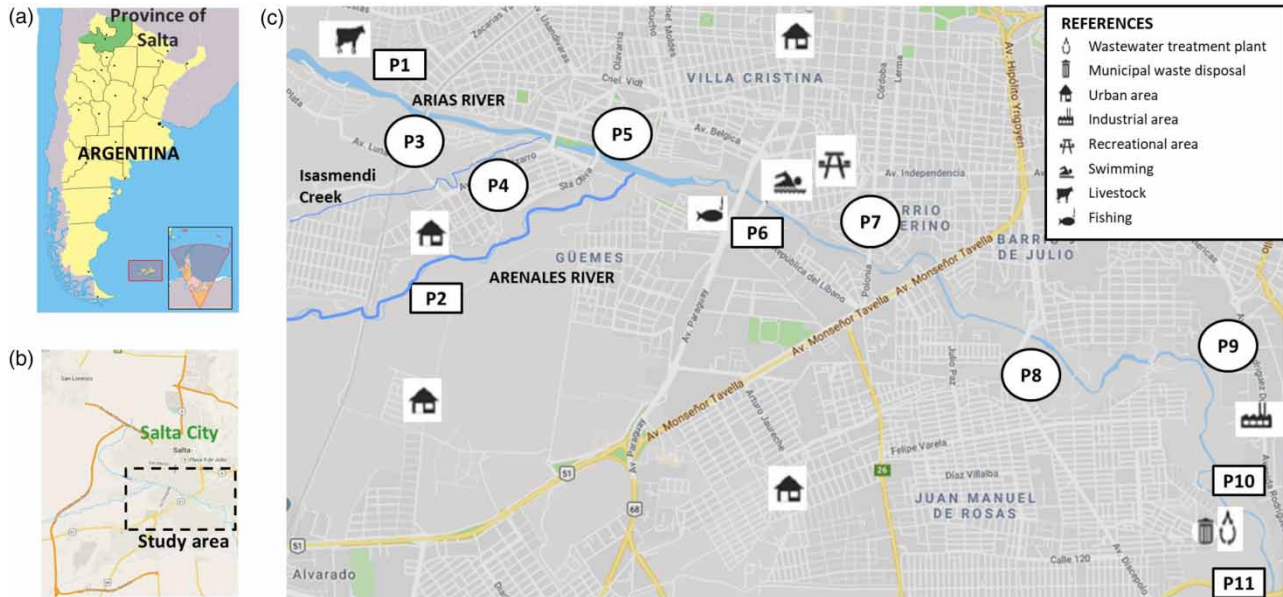
## METHODS

### Background

The city of Salta is the capital of the province of Salta, in northern Argentina. With a population of 783,323 inhabitants, it is located at the east of the Andes Mountains, in the Lerma Valley (INDEC 2010). The city is crossed by the Arias–Arenales River, which belongs to the Juramento–Salado watershed and runs west to east through a semi-rural area where the main use is for water supply, agricultural irrigation, recreational activities and livestock maintenance. When the river crosses the city, it receives pollution, such as illegal raw sewage, domestic and industrial effluents, illegal solids (deposits of domestic waste on the river banks) and many other untreated pollutants. Also, this river is subjected to seasonal fluctuations in water flow during the wet and dry seasons present in summer and winter, respectively (Poma *et al.* 2012).

### Environmental samples

A total of 116 water samples were collected during the period February 2009–February 2010 from the Arias–Arenales River. Eleven collection points (P1–P11) were used along the river where it runs through the city of Salta (12.5 km) (Figure 1). Briefly, P1 and P2 were selected as low-pollution controls on the Arias and Arenales rivers,



**Figure 1** | Geographical representation of the area under study: (a) province of Salta in Argentina, (b) area of study in Salta City and (c) monitoring points (P1–P11) along the Arias–Arenales River. The circles correspond to points on the river, while the rectangles correspond to points of discharges into the river. The main activities developed in them are also marked with the indicated icons.

respectively, before entering the city. Low thermotolerant coliform levels were determined in these sampling points all along the studied period (Poma *et al.* 2012). P3, P7, P8 and P9 are stormwater sources flowing into the river; P4 is on the Isasmendi Creek at the confluence with the Arias River, where a beef packing plant is located; P5 receives untreated sewage; P6 corresponds to a recreational area called Parque Los Sauces; and P10 and P11 are the upstream and downstream of the wastewater treatment plant and municipal landfill, respectively. One sample was collected per sampling point the first week of each month, during the morning. A 20 L water sample was collected per sampling point. Some points could not be monitored during the dry season because of the lack of flow. Therefore, a total of 13 water samples were collected in the sampling points P1, P2, P6, P7, P10 and P11; 12 samples in P8; 9 samples in P4; 6 samples in P3 and P5; and 5 samples in P9.

### Viruses concentration in water samples

The concentration procedure described previously by Rajal *et al.* (2007) was followed with small modifications (Poma *et al.* 2013). Briefly, water samples were spiked with PP7

(ATCC 15692-B2), bacteriophage of *P. aeruginosa*, to a final concentration of  $10^6$  genome copies (gc)/mL. Then, samples were concentrated 400× by ultrafiltration. The water samples were filtered through stainless steel sieves to remove solids, placed into the feed tank and pumped through an ultrafiltration system using a peristaltic pump. Two membrane units were used: Microza AHP 1010 (Pall Life Sciences, Port Washington, NY, USA) and Polyflux 24R (Gambro, Deerfield, IL, USA). Elution was performed using a 20 mL of a solution containing 0.05 M glycine/sodium hydroxide (pH 7.0) and 0.1% Tween 80. The final concentrated sample (approximately 50 mL) consisted of the eluate from the ultrafiltration unit plus the final retentate.

### Extraction of RNA and cDNA synthesis

Viral nucleic acids were extracted using the QIAamp Viral RNA Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Extracted RNA was reverse-transcribed (RT) into cDNA using random hexamer primers and avian myeloblastosis virus reverse transcriptase (Invitrogen, CA, USA).

## IPC detection

The detection of PP7 was performed by qPCR using the ABI 7500 Real-Time PCR System (Applied Biosystems, CA, USA) and the Sequence Detection Software version 1.0 (Applied Biosystems). The qPCR reaction was performed in a final volume of 25  $\mu$ L by using TaqMan Universal PCR Master Mix (Applied Biosystems) and primers and probe designed by Rajal *et al.* (2007).

## RVA detection and genotype characterization

cDNA products were used as templates for 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). The amplified products of the first RT-PCR rounds were used as templates for G and P genotyping by multiplex heminested PCR. For G genotyping, G1 to G4, G8 and G9-specific primers (Gouvea *et al.* 1990) were used, and P[4], P[6], P[8], P[9] and P[10]-specific primers were used for P genotyping (Gentsch *et al.* 1992; Iturriza-Gomara *et al.* 2000). The amplicons were analyzed by electrophoresis on 10% polyacrylamide gels and visualized after silver staining (Herring *et al.* 1982).

## Quantification of RVA-positive samples

The rotavirus load was determined in RVA-positive water samples in duplicate by qPCR using the ABI 7500 Real-Time PCR System (Applied Biosystems) and the Sequence Detection Software version 1.0 (Applied Biosystems). The qPCR reaction was performed in a final volume of 25  $\mu$ L by using Environmental PCR Master Mix (Applied Biosystems) and primers and probe designed by Zeng *et al.* (2008). A standard curve ( $10^6$ – $10^1$  copies per reaction) was generated using tenfold serial dilutions of a plasmid construction of pCR<sup>®</sup> 2.1-TOPO<sup>®</sup> vector (Invitrogen, USA) containing the NSP3 gene region of RVA that yield a slope of  $-3.59$  and a reaction efficiency of 0.90. All molecular procedures included non-template controls as well as positive controls that were run simultaneously, and separated rooms were used to avoid cross-contamination. 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. Concentrations were defined as the average of the duplicate data obtained. The limits of detection (LOD) and quantification (LOQ) of the qPCR were five copies per reaction.

## RESULTS AND DISCUSSION

The IPC PP7 was detected in all the water samples analyzed, with a recovery range between 1.5% and 29.7%, revealing that no sample showed total inhibition of PCR and that there was no total loss of the viral particles at any point in the process. RVA genome was detected in 4/116 samples (3.4%, 95% CI: 0.1–7.0%), at a mean concentration of  $6.51 \times 10^5$  gc/L ( $1.9 \times 10^5$ – $3.8 \times 10^6$ ). The viral load could not be determined in one of the RVA-positive samples (Table 1).

Previous studies of the Arias–Arenales River showed the variable detection frequencies of enteric viruses: 45% adenovirus (concentration range:  $2.6 \times 10^4$ – $2.6 \times 10^7$  gc/L), 9% norovirus ( $3.1 \times 10^4$ – $6.9 \times 10^5$  gc/L), 8% enterovirus ( $5.1 \times 10^4$ – $1.8 \times 10^6$  gc/L; Poma *et al.* 2012), 6% hepatitis A virus (Blanco Fernandez *et al.* 2012) and 0.02% hepatitis E virus (Pisano *et al.* 2018). In this sense, RVA showed similar detection frequencies and viral load to other enteric viruses, while adenovirus revealed a pattern of pollution very different from the other viruses. The occurrence of RVA in the Arias–Arenales River was lower than in surface waters of other cities from Argentina, such as Córdoba, where RVA was detected in 18.7–100% of the surface waters tested, with a viral load ranging from  $1.9 \times 10^3$  to  $8.6 \times 10^6$  gc/L (Prez *et al.* 2015).

Also, RVA frequency in the Arias–Arenales River was lower than in surface water from different countries. RVA was detected in 90% of the river water samples tested in

**Table 1** | Rotavirus detection in the Arias–Arenales River, in northwest Argentina

Monitoring site	Month (season)	RVA genotype	RVA load (gc/L)
P2	May (dry season)	G1P[8]	$3.8 \times 10^6$
P6	April (wet season)	G9P[8]	$1.9 \times 10^5$
P7	April (wet season)	G9P[8]	$3.8 \times 10^5$
P8	March (wet season)	G4P[8]	NQ

NQ, not quantifiable.

Germany, with viral loads ranging from  $1.2 \times 10^1$  to  $4.2 \times 10^4$  gc/L (Hamza *et al.* 2009). In Amazonia, Brazil, RVA was the most common enteric virus detected in 44.2% of the river water samples analyzed (Miagostovich *et al.* 2008). In a river basin in Minas Gerais, Brazil, RVA was detected in 62.5% of the water samples (Assis *et al.* 2015), while it was detected in 17.5% of river water samples from Slovenia (Steyer *et al.* 2011).

RVA strains from the Arias–Arenales River were characterized as G1P[8], G4P[8] and G9P[8] (Table 1), which are common genotypes circulating in the human population (Durmaz *et al.* 2014; Collins *et al.* 2015). An epidemiological study carried out in Argentina by Degiuseppe *et al.* (2013) reported the detection of G1P[8], G3P[8] and G12P[8] genotypes in the northern region of Argentina in 2009, while G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] were found in 2010 (Degiuseppe *et al.* 2013). These results indicate that the viral variants detected in the surface waters of Salta match with genotypes reported in clinical cases of the region and the world (Santos *et al.* 2016; Tian *et al.* 2018; Tapisiz *et al.* 2019), which infers that the RVA species excreted by the infected people could be contaminating the river waters and also the Arias–Arenales River would be a potential water source of RVA infection.

Positive samples for RVA were collected from a sampling point selected as low-pollution control on the Arenales River, before the river enters the city (P2), a river point located on the Arias–Arenales River which is a recreational area called Parque Los Sauces (P6) and from two stormwater sources flowing into the river (P7 and P8) (Table 1). The monitoring sites located downstream the discharges of sewage water (treated and untreated) did not reveal RVA contamination (P5 and P11). Neither P10, which is located downstream the discharge of the channels P7 and P8 into the river, showed rotavirus contamination. RVA was not detected in the channels P3, P4 and P5, which discharge their waters into the river, revealing that none of them seemed to be the cause of rotavirus pollution of the monitoring point P6, situated in the river course.

No seasonality in RVA detection was noted in the Arias–Arenales River (Table 1). However, due to the small number of samples that tested positive for RVA, the available data does not warrant a discussion of seasonal variation in RVA prevalence.

There is a growing concern about the levels of fecal contamination in surface waters due to spills from point sources of pollution (Rusinol *et al.* 2014). In the present study, two stormwater discharges (P7 and P8) flowing into the river resulted from point sources of RVA contamination. These two stormwater sources run through the city, receiving the impact of the populations settled on its banks, but suffer little impact of agricultural activities. RVA was not detected in the monitoring sites located downstream the discharge of treated (P11) and untreated sewage water (P5), which in previous studies showed the worst microbiological quality (Poma *et al.* 2012). This could be related to the number of inhabitants of the city of Salta and the number of households that have access to sewers and also to the size and flow of the river.

In addition to the identified point sources of contamination, non-point sources or other non-identified sources of pollution were present in the Arias–Arenales River (P2 and P6), which can originate from runoff from agricultural areas that drain into rivers, urban surface runoff or waste washed by the wind into bodies of surface water, all of which can exhibit high microbial loads (Rusinol *et al.* 2014). The pollution from a non-point source can be the product of the contribution of many different sources and without a specific solution to solve the problem, making it difficult to regulate.

The identification of monitoring points contaminated by RVA alerts on the importance of maintaining environmental sanitation in the city (in order to avoid RVA presence in storm drains) and in recreational areas. In this sense, environmental virology can contribute with the microbiological care of surface waters still little impacted by anthropogenic activities, helping with effective strategies linked to the control and prevention of water-related diseases and the preservation of the environment.

## CONCLUSIONS

The Arias–Arenales River presented unusual and sporadic contamination by RVA, originated from stormwater discharges and a variety of non-identified sources, and support the essential need of viral indicators for enhanced monitoring of water quality.



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