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ORIGINAL PAPER

Persistent Detection of Cosavirus and Saffold Cardiovirus in Riachuelo River, Argentina

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Abstract

Cosaviruses (CoSV) and Saffold cardiovirus (SAFV) are novel members of the *Picornaviridae* family. The Matanza-Riachuelo river basin covers a total area of 2200 km^2 with approximately 60 km long. Its last section is called Riachuelo River. The aim of this study was to describe the circulation of both picornaviruses and their relationship with the environmental situation of the Riachuelo River using 274 samples collected from 2005 to 2015. CoSV and SAFV were investigated in samples available by two periods: 2005–2006 and 2014–2015 (103 and 101, respectively). Physicochemical and bacteriological parameters confirmed very high levels of human fecal contamination during the 11 years evaluated. CoSV was detected in 85.7% (66/77) and 65.4% (17/26) of the samples collected in 2005–2006 and 2014–2015 periods, respectively. Species A and D were identified, the first one being widely predominant: 74.1% (20/27) and 75.0% (3/4) in both periods. SAFV virus was detected in 47.1% (32/68) and 52.6% (10/19) in periods 2005–2006 and 2014–2015, respectively. SAFV-6 was the most identified genotype in the entire study, while SAFV-3 was predominant in 2005–2006. The contribution of genotypes 1, 2, 4 and 8 was minor. The high prevalence of CoSV and SAFV suggests that both viruses have been circulating in Argentina at least since 2005. Our results show that a watercourse with high rates of human fecal contamination can become a persistent source of new viruses which capacity to produce human diseases is unknown.

Keywords Cosavirus · Saffold · Cardiovirus · Riachuelo river

Introduction

The family *Picornaviridae* is one of the most genetically diverse families of positive-sense single-stranded RNA viruses. Currently, it consists of 110 species grouped into 47 genera being in continuous expansion (Zell et al. 2017). Seven genera cause a broad spectrum of diseases in humans in developed countries: *Enterovirus, Parechovirus, Hepatovirus, Kobuvirus, Salivirus, Cardiovirus and Cosavirus*.

Cosaviruses (CoSV) were identified in 2008 in stool samples of children with acute flaccid paralysis (AFP) in Pakistan and Afghanistan (Kapoor et al. 2008b). They are classified into 5 species called: A, B, D, E and F. In addition, CoSV have been detected in 1.5–3.5% of patients with acute gastroenteritis in Thailand, China and Tunisia (Menage et al. 2017; Yu et al. 2017; Ayouni et al. 2016). In the environment, CoSV was found in 25–71% of raw sewage samples collected in Italy, Japan and United States (Bonanno Ferraro et al. 2018; Haramoto and Otagiri 2014; Kitajima et al. 2015).

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Cardioviruses are classified into six species called A-F (https://talk.ictvonline.org/). The species cardiovirus D includes the Saffold virus (SAFV) which affects humans (Zell et al. 2017). Partial sequencing of VP1 gene allowed its classification in 11 genotypes (SAFV1-11) (Tan et al. 2017). All SAFV types have been associated with gastroenteritis, often in co-infections with other known viral pathogens (Dapra et al. 2018; Li et al. 2017). Additionally, SAFV has been detected in 0.4% to 9.3% of children suffering from respiratory infections (Itagaki et al. 2018; Zhang et al. 2015; Lin et al. 2015). In Japan, SAFV was detected in 16.7% of the raw sewage samples collected between 2015 and 2016 (Thongprachum et al. 2018). Another study conducted in the United States evidenced the presence of 43% of this virus in untreated sewage (Blinkova et al. 2009).

In South America, there are few reports describing the circulation of these picornaviruses. CoSV was detected in 3.6% of Brazilian children with gastroenteritis and in 33.8% of their healthy control cases (Stocker et al. 2012). In Peru, SAFV-3 was identified in a child with diarrhea and respiratory illness (Leguia et al. 2015). In addition, in Brazil SAFV-2 was detected in 1.6% of stool samples (Drexler et al. 2008). Finally, two studies report the simultaneous detection of both picornaviruses. In Bolivia, SAFV-1 was the most common type identified, followed by SAFV-2, SAFV-4, and SAFV-9 and a higher diversity of CoSV species was found in stool samples obtained from AFP cases (Nix et al. 2013). In Venezuela, metagenomics techniques identified complete genomes of SAFV-1, 2 and 9 and a recombinant CoSV E/D in feces from indigenous population (Siqueira et al. 2018).

The Matanza Riachuelo River Basin (CMR) covers a total area of 2200 km^2, its length being approximately 60 km. It includes a large area within the Autonomous City of Buenos Aires and 14 municipalities of the province of Buenos Aires. The Matanza River lowest section is called Riachuelo. Its length is 15 km (ACUMAR 2018). Since 2001, Prefectura Naval Argentina, (PNA), the Argentine Coast Guard, has been periodically monitoring the environmental quality by measuring physicochemical and bacteriological parameters. The results show a high level of human and animal contamination that exceeds the allowed values for recreational waters or those recommend for protection of biota. Additionally, virological studies conducted between 2005 and 2006 showed a high prevalence of enterovirus (Cisterna et al. 2008), norovirus (Fernandez et al. 2012) and hepatitis A virus (Blanco Fernandez et al. 2012). In the present study, we describe the detection and circulation of these new picornaviruses in the Riachuelo River and their relation to environmental conditions.

Materials and Methods

Research Area

Data from the 2010 census showed that more than 8 million people live in CMR (https://www.indec.gob.ar/). It is the most urbanized and industrialized area of Argentina, with a high population density. At La Noria Bridge, the Matanza River changes its name to Riachuelo. The main causes of pollution are industrial and residential discharges. The sediments of the river are contaminated with high levels of heavy metals and toxic organic compounds (https://www.bdh.acumar.gov.ar/bdh3/index_contenido.php). The main tributary of the Riachuelo River is the Cildañez stream.

Environmental Samples

Since 2001, PNA has periodically and systematically sampled the Riachuelo Riverin four points: Pueyrredón bridge (PUE), Uriburu bridge (URI), Cildañez stream (CIL) and La Noria bridge (LNR) (Fig. 1). The physicochemical and bacteriological data from 274 water samples obtained between 2005 and 2015 were used to assess its environmental situation. Two variables associated with human fecal contamination (ammonia, and thermo-tolerant coliform count, TtC) and one that contributes to viral persistence (chemical oxygen demand, COD) were selected (Pinon and Vialette 2018). One liter of river water was collected in the morning at 50 cm depth in a sterile container to avoid contamination. The samples were stored at 4 °C and immediately transported to the laboratory for processing.

To investigate the presence of CoSV, 26 samples were used, collected between 2014 and 2015 during the monitoring activity. Furthermore, a retrospective investigation of this virus was carried out on 77 samples taken between 2005 and 2006. Cardiovirus was investigated, using 25 and 76 samples available in each studied period, respectively. Additionally, the presence of enteroviruses (EV) was investigated as a viral indicator of human fecal contamination using a total of 112 samples.

Sample Processing

Viruses were concentrated by viral adsorption-elution to a negatively charged membrane following Standard Methods with minor modifications (APHA et al. 2017). Briefly, the pH and the $MgCl_2$ level of one liter of water were adjusted to pH 3.5 and 0.01 N $MgCl_2$ by adding 1.0 N HCl and 5.0 N $MgCl_2$ solutions. Each sample was then filtered through a negatively charged 0.8 µm pore-sized nitrocellulose filter (47 mm in diameter, Millipore Corporation, Bedford, Mass.)

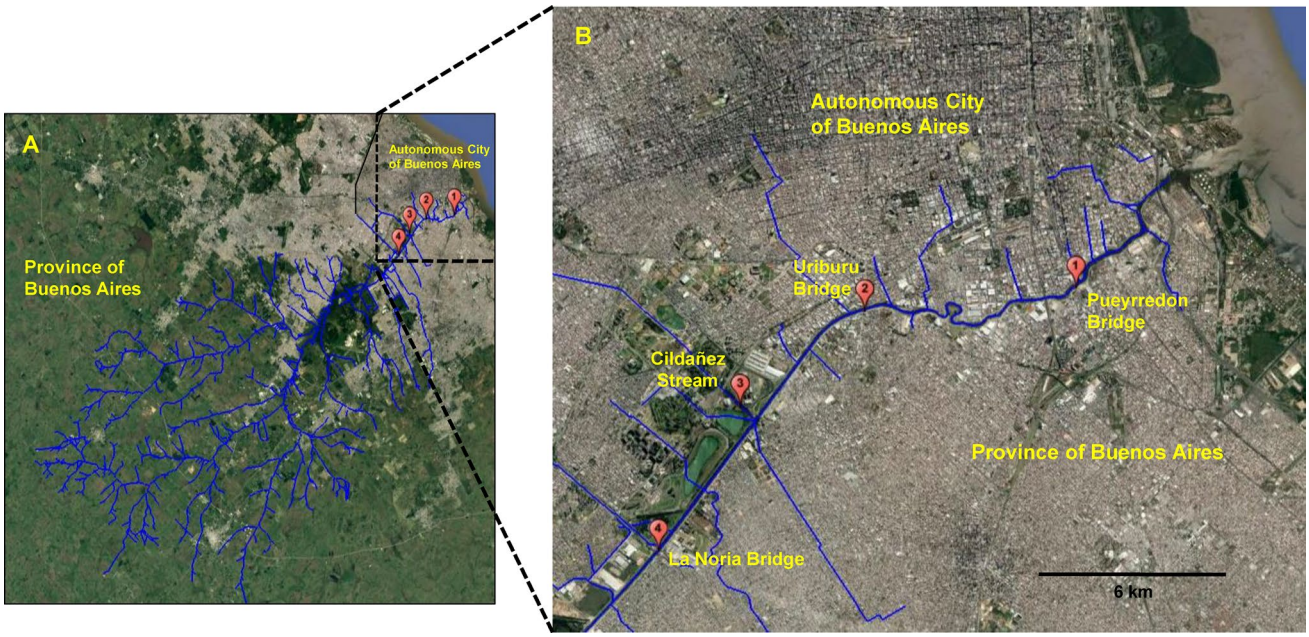

Fig. 1 A. Hydrographic network of the Matanza-Riachuelo Basin. B. Sampling sites on the Riachuelo River. Both figures were built using Google Earth and resources available in ACUMAR (https://mapas.acumar.gov.ar/datos/)

at a rate not exceeding 30 ml/min/cm^2, washed with 0.14 N NaCl and eluted with 0.05 N glycine-OHNa buffer (pH 11.5). The concentrates were stored in aliquots at − 80 °C. The NucliSens extraction kit (BioMerieux, France) was used for extraction and purification of nucleic acid according to the manufacturer's instructions. The eluted RNA (100 µl) was aliquoted and stored at − 80 °C until molecular analysis.

Virus Detection and Typing

Cardioviruses and cosaviruses were tested by genus-specific Taqman real-time RT-PCR assays (RT-qPCR) targeting the 5′ non-translated region (5′NTR) as previously described (Nix et al. 2013). The cardiovirus assay detects all Cardiovirus B and D, with the exception of genet fecal theilovirus. Detection and further genotyping of SAFV virus was performed in positive samples for cardiovirus test using a nested RT-PCR assay (nRT-PCR) targeting a portion of the genome encoding the VP1 capsid protein as previously described (Itagaki et al. 2011). Species identification of CoSV was carried out using a nRT-PCR as described by Kapoor (Kapoor et al. 2008a). EV RNA was detected using RT-qPCR previously described (Selvaraju et al. 2013). Amplicon PCR fragments were purified using the enzyme ExoSAP-IT® (Applied Biosystems), following manufacturer's instructions. Subsequently, both sense and antisense sequences were read using the BigDye® Terminator Cycle Sequencing v3.1 reagent (Applied Biosystems). Post sequencing purification was performed with the BigDye® X-Terminator™ commercial kit (Applied Biosystems) sequenced in an ABI3500 Genetic Analyzer (Hitachi, Applied Biosystems) automatic sequencer. Virus sequences were aligned with the Clustal W multiple alignment application using BioEdit v.7.1.9 sequence alignment editor program (Hall 1999). Identification of SAFV genotype or CoSV species was performed by phylogenetic analysis including representative sequences with their corresponding GenBank accession number. Phylogenetic trees were inferred using the Maximum Likelihood method based on the Tamura-3-parameter nucleotide substitution model with a discrete gamma distribution using MEGA6 Package (Tamura et al. 2013). Bootstrap values were calculated from 1000 replicates. The nucleotide sequences described herein have been deposited in the GenBank sequence database, accession No MT 211907-MT 211948 and MT 228084-MT 228114.

Statistical Analysis

We used Microsoft Excel descriptive tools. Data were analyzed using IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, N.Y., USA). Variability of the TtC, COD and ammonia parameters in relation to the place and year of collection was studied by analysis of variance (ANOVA) using data collected between 2005 and 2015. Differences in the values of the environmental variables between the considered periods (2004–2005 and 2014–2015) was studied by T Test and difference in the prevalence for the studied viruses was studied using chi square or Fisher test.

Furthermore, the association between the study viruses and the environmental variables collected in both periods were studied using binary logistic regression. In all cases, p values less than 0.05 were considered statistically significant.

Results

Four collection sites were used to assess the environmental quality of the Riachuelo River. There were no statistically significant differences for ammonia, COD and logTtC concentrations between the sampling sites analyzed ($p = 0.598$, 0.765 and 0.653, respectively). However, we observe a high variability for these three variables between the sampling years ($p = 0.000$). Nonetheless, 227 (92.7%) of the 245 ammonia measurements exceeded the recommended values of < 0.6 mg/L. Besides, 242 (97.2%) of the 249 COD values were higher than expected values of < 30 mg/L in surface waters (ACUMAR 2019). All logTtC values ($n = 235$) exceed the recreational water use accepted values ($\geq 2 \times 10^2$ NMP/100 ml; 2.3 log NMP/100 mL) (MSAL 2017) (Figure S1).

The comparison between the mean values of the environmental variables between 2005–2006 and 2014–2015 showed a decrease in the logTtC: 0.99 log NMP/100 mL 95% CI (0.62, 1.37), $p = 0.000$ and in the ammonia concentration: 5.59 mg/L 95% CI (3.37, 7.81), $p = 0.000$. Conversely, the difference between the average values of COD in both periods was not significant: 4.31 mg/L; 95% CI (− 6.33, 14.96), $p = 0.487$ (Fig. 2a–c). Finally, human enterovirus was detected in 83 of 86 (96.5%) and in 19 of 26 (73.1%) samples in both periods.

Cardiovirus was detected in 68 out of the 76 (89.5%) samples and in 19 out of the 25 (76.0%) samples obtained in 2005–2006 and 2014–2015, respectively, $p = 0.099$ (Table 1). Saffold virus was identified in 32 of 68 (47.1%) and 10 of 19 (52.6%) of the total positive samples in cardiovirus assay, $p = 0.067$. SAFV-6 was the most identified virus throughout the study while SAFV-3 was predominant in 2005–2006. Genotypes 1, 2, 4, and 8 were found in minority proportion (Fig. 3). CoSV were detected in 66 of 77 samples (85.7%) and in 17 of 26 samples (65.4%) collected in both periods, $p = 0.028$ (Table 1). CoSV species were identified in 27 of 76 (40.9%) and 4 of 25 (23.5%) of the total virus detected showing the presence of two species A and D (Fig. 4). Species A was widely predominant, accounting for 74.1% and 75.0% in both periods.

Considering the environmental variables such as ammonia, COD and TtC concentrations and EV prevalence, we observed that cardiovirus were associated with the presence of EV and high values of TtC while CoSV showed association only with enteroviruses. SAFV prevalence was not associated to any of the study variables (Table 2).

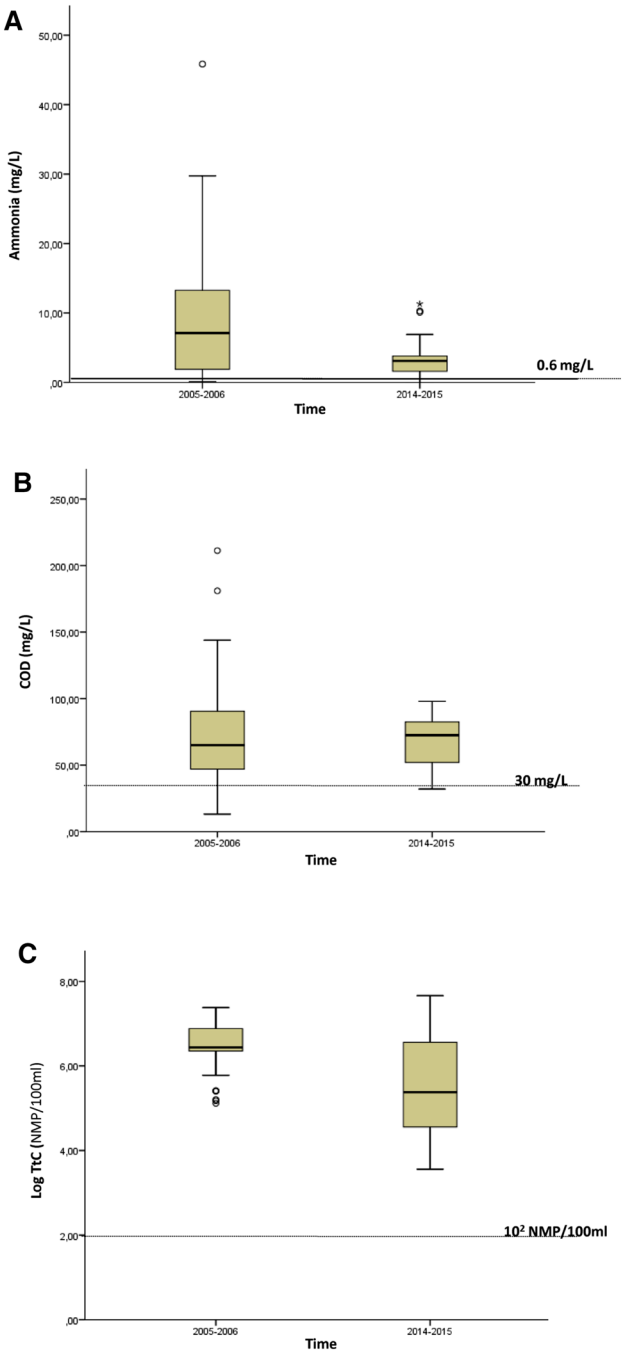

Fig. 2 Box plot showing the comparison between the environmental parameters average values between 2005–2006 and 2014–2015. **a** Ammonia, **b** COD, **c** TtC. The dotted line indicates the suggested or allowed values for each environmental variable. *TtC* thermotolerant coliforms count, *COD* chemical oxygen demand

Discussion

The Riachuelo River has a long history of human contamination, which suggested that it was a suitable place to find new picornaviruses. Analysis of these variables confirmed high

Table 1 Molecular detection and typing of picornavirus in Riachuelo River, Argentina

Virus	2005–2006	2014–2015	Total
Cardiovirus B	68/76 (89.5%)	19/25 (76.0%)	87/101 (86.1%)
SAFV	32/68 (47.1%)	10/19 (52.6%)	42/87 (48.3%)
SAFV-1	2/32 (6.3%)	0/10 (0.0%)	2/42 (4.8%)
SAFV-2	1/32 (3.1%)	2/10 (20.0%)	3/42 (7.1%)
SAFV-3	14/32 (43.8%)	0/10 (0.0%)	14/42 (33.3%)
SAFV-4	1/32 (3.1%)	0/10 (0.0%)	1/42 (2.4%)
SAFV-6	13/32 (40.6%)	8/10 (80.0%)	21/42 (50.0%)
SAFV-8	1/32 (3.1%)	0/10 (0.0%)	1/42 (2.4%)
CoSV	66/77 (85.7%)	17/26 (65.4%)	83/103 (80.6%)
CoSV-A	20/27 (74.1%)	3/4 (75.0%)	23/31 (74.2%)
CoSV-D	7/27 (25.9%)	1/4 (25.0%)	8/31 (25.8%)
EV	83/86 (96.5%)	19/26 (73.1%)	102/112 (91.1%)

SAFV Saffold virus, *CoSV* cosavirus, *EV* enterovirus

pollution levels in the river throughout the period under consideration. These results coincide with the multidimensional evaluation of the quality of surface water by Authority of the Matanza-Riachuelo Basin (ACUMAR), through the surface water quality index (ICA-Sup). This index (quantified from 0 to 100; very good–very poor) synthesizes 12 physicochemical and biological parameters. Between 2015 and 2016, the ICA-Sup showed values between 18.7 and 22.2 which shows a very poor average quality (https://www.acumar.gob.ar/indicadores/indice-calidad-agua-superficial-ica-sup).

Since 2005, PNA uses four sampling points for its environmental assessment. Our results shown that there were no significant differences between the four sampling sites. This is probably due to its short length and because it is rectified in its last section. This anthropic intervention of the river banks, a characteristic of urban aquatic ecosystems, implies a decrease in its flow, erosion of its margins and loss of riverine vegetation, which avoid the self-purification processes of the river and determine a reduction in the richness of biota and high concentrations of pollutants and bacteria (Booth et al. 2015).

Several authors have explored the integration of bacterial and viral indicators to improve water quality. Our results indicate a correlation between enteroviruses and coliforms and the presence of cardioviruses and cosaviruses. However, these results should be interpreted with caution since it would be necessary to carry out other studies expanding the number of samples, using methods that allow viral quantification and detecting other viral genetic markers such as adenovirus or norovirus to reach more sustainable conclusions (Marion et al. 2014; Texeira et al. 2020).

Since the creation of ACUMAR in 2008, real progress has been made that improves the environmental condition of the Riachuelo River. Several activities were carried out, such as the removal of vehicles, old docks and the remains of ships. In addition, 12,000 stalls were relocated on the banks of the river (ACUMAR 2018). The reduction noted in the parameters associated with human contamination, including the prevalence of enteroviruses, between the two periods under analysis could relate to the above mentioned improvement actions. Nevertheless, the investigation of new picornaviruses evidenced discordant results. CoSV showed a reduction in the second period while SAFV did not present the same variation. These differences could stem from the dissimilar technical strategies used for the investigation of each virus. CoSV detection was performed by RT-qPCR. As mentioned by other authors, this technique is less susceptible to the usual inhibition phenomena present in environmental samples than nested PCR assays like the one used for SAFV detection (Uchii et al. 2019). An additional contribution to this disagreement could be related to cardiovirus assay, which also detects animal cardioviruses and could have influenced its detection rate. In fact, the sequencing of the RT-qPCR product suggested the presence of Theiler's Murine Encephalomyelitis Virus (TMEV) in some samples using NCBI BLAST (data not shown). More thorough studies should be carried out to understand the relationship between the Riachuelo River environmental parameters and enteric viruses.

The detection rate of the Saffold virus found in our study was similar to the range reported (35.7–43%) by other authors in samples of river or sewage discharge (Aminipour et al. 2020). Furthermore, a great diversity of SAFV was found in Riachuelo River, including six different genotypes. The time frame analyzed revealed co-circulation of multiple SAFV genotypes similar to that described in enteroviruses epidemiology. SAFV-2 and SAFV-3 represented 40% of the identified genotypes. Both have been associated in numerous studies with upper respiratory disease in children, therefore they should be included in the differential diagnoses (Itagaki et al. 2018; Lindner et al. 2019). Additionally, SAFV-6 was predominantly identified in 2014–2015. Interestingly, the same genotype emerged in 2016 in Thailand, although its health impact is unknown (Menage et al. 2017).

Most of the environmental studies that refer to the detection of CoSV have been carried out in sewage or treatment plants. The reported detection range is extremely diverse (16.2–71%) depending on the molecular technique used (Bonanno Ferraro et al. 2018; Kitajima et al. 2015; Haramoto and Otagiri 2014). In our study, the RT-qPCR assay showed the highest values (65.4–85.7%) unlike nested PCR, that needs to amplify longer fragments to genotype, which can result in lower sensitivity (Beyer et al. 2020). A high proportion of the cosaviruses identified in Riachuelo River were identified as species A resembling that reported worldwide (Kitajima et al. 2015). In South America, CoSVs identified in stool samples have

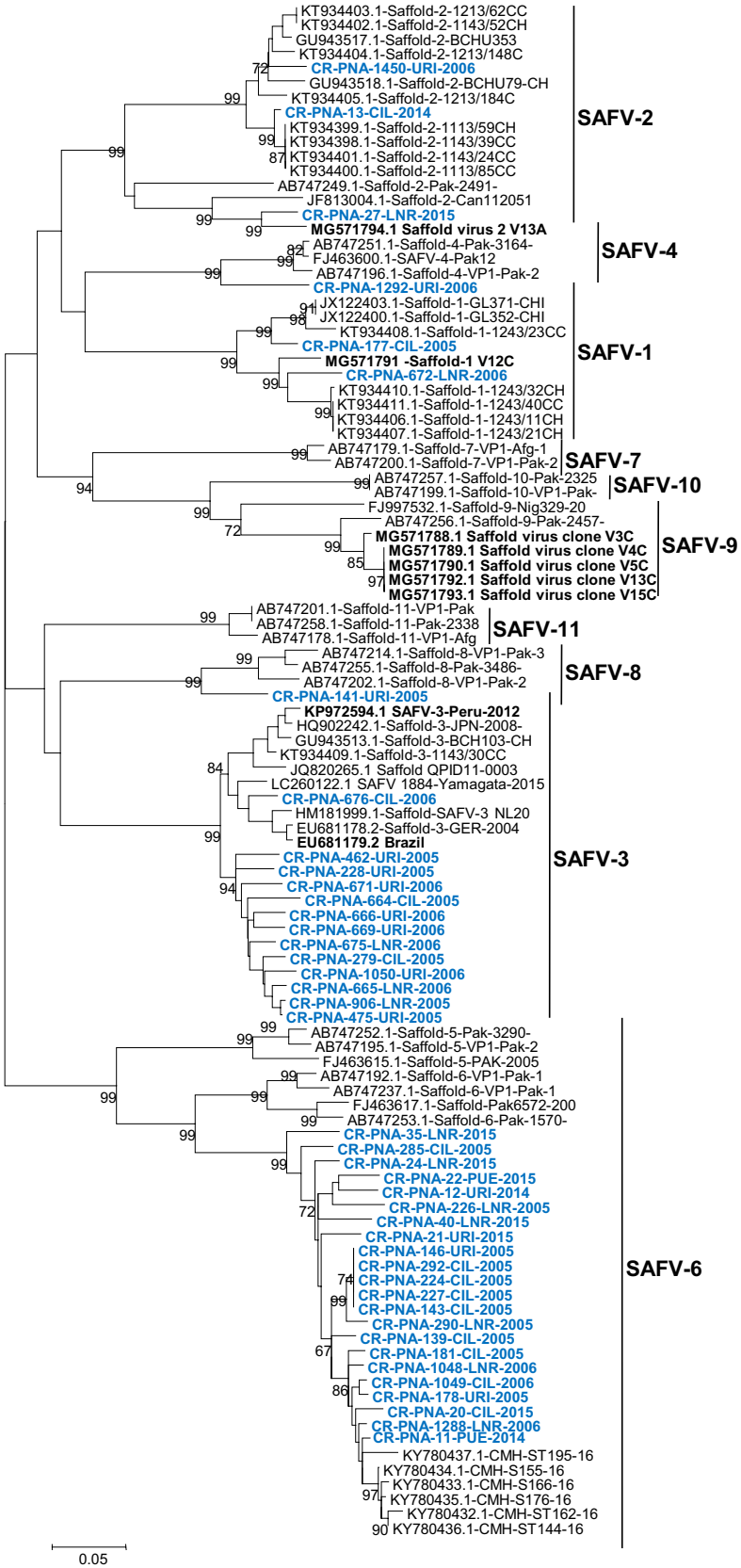

Fig. 3 Phylogenetic tree of SAFV detected in Riachuelo River, partial VP1 region (267 nt). This study's strains are shown in blue. SAFV genotypes are shown in bold (Color figure online)

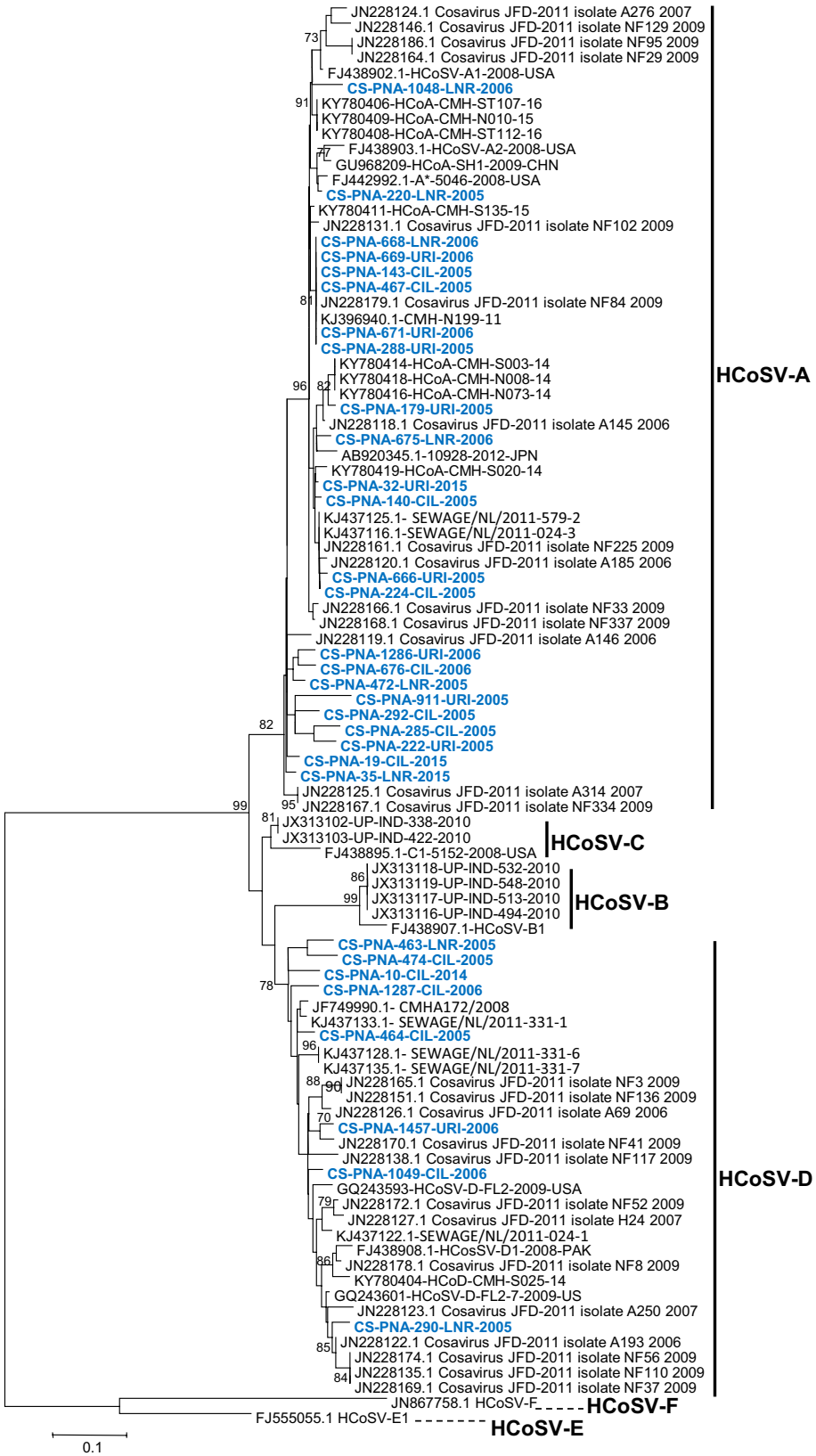

Fig. 4 Phylogenetic tree of CoSV detected in Riachuelo River, 5′ UTR region (279 nt). This study's strains are shown in blue. HCoSV species are shown in bold (Color figure online)

Table 2 Association between picornaviruses and the environmental variables in Riachuelo River, Argentina

Picornaviruses	OR (95% CI)	*p*
Cardiovirus ($n=101$)		
logTtC	2.43 (1.20–4.94)	0.014
DQO (mg/l)	1.01 (0.99–1.03)	0.391
Ammonia (mg/l)	1.05 (0.94–1.17)	0.368
EV (%)	15.56 (3.62–66.91)	0.000
SAFV ($n=87$)		
logTtC	0.88 (0.47–1.65)	0.685
DQO (mg/l)	1.00 (0.99–1.01)	0.864
Ammonia (mg/l)	0.99 (0.93–1.08)	0.979
EV (%)	2.93 (0.29–29.32)	0.361
CoSV ($n=103$)		
logTtC	1.56 (0.86–2.83)	0.146
DQO (mg/l)	1.00 (0.99–1.02)	0.949
Ammonia (mg/l)	1.05 (0.96–1.16)	0.294
EV (%)	5.20 (1.34–20.20)	0.017

SAFV Saffold virus, *CoSV* cosavirus, *EV* enterovirus

been widely diverse, suggesting that their prevalence is highly variable geographically and temporally resulting in a more complex epidemiology (da Costa et al. 2018; Siqueira et al. 2018). Other studies involving a larger geographical representation should be carried out to identify viral circulation patterns in our country.

One of the limitations of our study is that we have not included a control of the viral concentration and/or inhibition process of the PCR assays. Although, the high detection rate of EV would suggest that there was no interference in the viral investigation, process controls such as bacteriophage PP7 or mengovirus (Poma et al. 2013; Londone-Bailon and Sanchez-Robinet 2018), should be included to determine precise values of prevalence of these new picornaviruses.

According to our results, it is clear that a river exhibiting high rates of human fecal contamination can become a source of persistent detection of new viruses with unknown capability to produce human diseases. High prevalence of CoSV and SAFV suggests that both have been circulating in the population at least since 2005 in Argentina. Further studies should be carried out to characterize their spread, diversity and clinical importance in the South American Region.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

ACUMAR. (2018). *Facing the river. Participatory proposal towards a territorial strategic plan for the Matanza Riachuelo River Basin*. Buenos Aires: ACUMAR; Ministerio del Interior, Obras Publicas y Vivienda.

ACUMAR. (2019). *RESOL-2019-283-APN-ACUMAR#MI*. Buenos Aires, Argentina: Ministerio de Ambiente y Desarrollo Sustentable.

Aminipour, M., Ghaderi, M., & Harzandi, N. (2020). First occurrence of saffold virus in sewage and river water samples in Karaj, Iran. *Food and Environmental Virology, 12*(1), 75–80. https://doi.org/10.1007/s12560-019-09415-y

APHA, Awwa, & WEF. (2017). Virus concentration from small sample volumes by adsorption to and elution from microporous filters. In A. P. H. Association (Ed.), *Standard method for examination of water and waste waters* (pp. 1287–1304). Washington: American Pub Health Association.

Ayouni, S., Estienney, M., Hammami, S., Neji Guediche, M., Pothier, P., Aouni, M., et al. (2016). Cosavirus, salivirus and bufavirus in diarrheal Tunisian infants. *PLoS ONE, 11*(9), e0162255. https://doi.org/10.1371/journal.pone.0162255

Beyer, S., Szewzyk, R., Gnirss, R., Johne, R., & Selinka, H. C. (2020). Detection and characterization of hepatitis E Virus genotype 3 in wastewater and urban surface waters in Germany. *Food and Environmental Virology, 12*(2), 137–147. https://doi.org/10.1007/s12560-020-09424-2

Blanco Fernandez, M. D., Torres, C., Riviello-Lopez, G., Poma, H. R., Rajal, V. B., Nates, S., et al. (2012). Analysis of the circulation of hepatitis A virus in Argentina since vaccine introduction. *Clinical Microbiology & Infection, 18*(12), E548-551. https://doi.org/10.1111/1469-0691.12034

Blinkova, O., Rosario, K., Li, L., Kapoor, A., Slikas, B., Bernardin, F., et al. (2009). Frequent detection of highly diverse variants of cardiovirus, cosavirus, bocavirus, and circovirus in sewage samples collected in the United States. *Journal of Clinical Microbiology, 47*(11), 3507–3513. https://doi.org/10.1128/JCM.01062-09

Bonanno Ferraro, G., Mancini, P., Divizia, M., Suffredini, E., Della Libera, S., Iaconelli, M., et al. (2018). Occurrence and genetic diversity of human cosavirus in sewage in Italy. *Food and Environmental Virology*. https://doi.org/10.1007/s12560-018-9356-2

Booth, D. B., Roy, A. H., Smith, B., & Capps, K. A. (2015). Global perspectives on the urban stream syndrome. *Freshwater Sience, 35*(1), 412–420. https://doi.org/10.1086/684940

Cisterna, D., Lema, C., Riviello López, G., Freyre, L., Martinez, L., Torres, C., et al. (2008). Detección y caracterización de enterovirus y hepatitis A en el Riachuelo y Río de la Plata. *Revista Argentina Microbiologia, 40*(Supl. 1), 49–50.

da Costa, A. C., Luchs, A., Milagres, F. A. P., Komninakis, S. V., Gill, D. E., Lobato, M., et al. (2018). Near full length genome of a recombinant (E/D) cosavirus strain from a rural area in the central region of Brazil. *Scientific Reports, 8*(1), 12304. https://doi.org/10.1038/s41598-018-30214-1

Dapra, V., Montanari, P., Rassu, M., Calvi, C., Galliano, I., & Bergallo, M. (2018). Prevalence of human cosavirus and saffold virus in young children with gastroenteritis, Northern Italy. *Minerva Pediatrica*. https://doi.org/10.23736/S0026-4946.18.05219-2.

Drexler, J. F., Luna, L. K., Stocker, A., Almeida, P. S., Ribeiro, T. C., Petersen, N., et al. (2008). Circulation of 3 lineages of a novel Saffold cardiovirus in humans. *Emerging Infectious Diseases, 14*(9), 1398–1405. https://doi.org/10.3201/eid1409.080570

Fernandez, M. D., Torres, C., Poma, H. R., Riviello-Lopez, G., Martinez, L. C., Cisterna, D. M., et al. (2012). Environmental surveillance of norovirus in Argentina revealed distinct viral diversity patterns, seasonality and spatio-temporal diffusion processes. *Science of the Total Environment, 437,* 262–269. https://doi.org/10.1016/j.scitotenv.2012.08.033

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series, 41,* 111–120.

Haramoto, E., & Otagiri, M. (2014). Occurrence of human cosavirus in wastewater and river water in Japan. *Food and Environmental Virology, 6*(1), 62–66. https://doi.org/10.1007/s12560-013-9120-6

Itagaki, T., Abiko, C., Aoki, Y., Ikeda, T., Mizuta, K., Noda, M., et al. (2011). Saffold cardiovirus infection in children associated with respiratory disease and its similarity to coxsackievirus infection. *The Pediatric Infectious Disease Journal, 30*(8), 680–683. https://doi.org/10.1097/INF.0b013e31821608a8

Itagaki, T., Aoki, Y., Matoba, Y., Tanaka, S., Ikeda, T., Matsuzaki, Y., et al. (2018). Detection of Saffold viruses from children with acute respiratory infections in Yamagata, Japan, between 2008 and 2015. *Journal of Medical Virology, 90*(1), 34–40. https://doi.org/10.1002/jmv.24928

Kapoor, A., Victoria, J., Simmonds, P., Slikas, E., Chieochansin, T., Naeem, A., et al. (2008). A highly prevalent and genetically diversified Picornaviridae genus in South Asian children. *Proceedings of the National Academy of Sciences of the United States of America, 105*(51), 20482–20487. https://doi.org/10.1073/pnas.0807979105

Kapoor, A., Victoria, J., Simmonds, P., Wang, C., Shafer, R. W., Nims, R., et al. (2008). A highly divergent picornavirus in a marine mammal. *Journal of Virology, 82*(1), 311–320. https://doi.org/10.1128/JVI.01240-07

Kitajima, M., Rachmadi, A. T., Iker, B. C., Haramoto, E., Pepper, I. L., & Gerba, C. P. (2015). Occurrence and genetic diversity of human cosavirus in influent and effluent of wastewater treatment plants in Arizona, United States. *Archives of Virology, 160*(7), 1775–1779. https://doi.org/10.1007/s00705-015-2435-x

Leguia, M., Loyola, S., Rios, J., Juarez, D., Guevara, C., Silva, M., et al. (2015). Full genomic characterization of a saffold virus isolated in Peru. *Pathogens, 4*(4), 816–825. https://doi.org/10.3390/pathogens4040816

Li, L. L., Liu, N., Yu, J. M., Ao, Y. Y., Li, S., Stine, O. C., et al. (2017). Analysis of Aichi virus and Saffold virus association with pediatric acute gastroenteritis. *Journal of Clinical Virology, 87,* 37–42. https://doi.org/10.1016/j.jcv.2016.12.003

Lin, T. L., Lin, T. H., Chiu, S. C., Huang, Y. P., Ho, C. M., Lee, C. C., et al. (2015). Molecular epidemiological analysis of Saffold cardiovirus genotype 3 from upper respiratory infection patients in Taiwan. *Journal of Clinical Virology, 70,* 7–13. https://doi.org/10.1016/j.jcv.2015.06.100

Lindner, K., Ludwig, M., Bootz, F., Reber, U., Safavieh, Z., Eis-Hubinger, A. M., et al. (2019). Frequent detection of Saffold cardiovirus in adenoids. *PLoS ONE, 14*(7), e0218873. https://doi.org/10.1371/journal.pone.0218873

Londone-Bailon, P., & Sanchez-Robinet, C. (2018). Efficiency evaluation of the process control virus "Mengovirus" in real time RT-PCR viral detection in the bivalve mollusc *Donax* sp. *Journal of Virological Methods, 262,* 20–25. https://doi.org/10.1016/j.jviromet.2018.09.006

Marion, J. W., Lee, C., Lee, C. S., Wang, Q., Lemeshow, S., Buckley, T. J., et al. (2014). Integrating bacterial and viral water quality assessment to predict swimming-associated illness at a freshwater beach: a cohort study. *PLoS ONE, 9*(11), e112029. https://doi.org/10.1371/journal.pone.0112029

Menage, L., Yodmeeklin, A., Khamrin, P., Kumthip, K., & Maneekarn, N. (2017). Prevalence of human cosavirus and saffold virus with an emergence of Saffold virus genotype 6 in patients hospitalized with acute gastroenteritis in Chiang Mai, Thailand, 2014–2016. *Infection, Genetics and Evolution, 53,* 1–6. https://doi.org/10.1016/j.meegid.2017.05.005

MSAL. (2017). Directrices sanitarias para uso seguro de aguas recreativas *Módulo II: Directrices sanitarias para enteropatógenos y microorganismos oportunistas en agua ambiente*. Buenos Aires: Departamento de Salud Ambiental, Dirección Nacional de Determinantes de la Salud.

Nix, W. A., Khetsuriani, N., Penaranda, S., Maher, K., Venczel, L., Cselko, Z., et al. (2013). Diversity of picornaviruses in rural Bolivia. *Journal of General Virology, 94*(Pt 9), 2017–2028. https://doi.org/10.1099/vir.0.053827-0

Pinon, A., & Vialette, M. (2018). Survival of viruses in water. *Intervirology, 61*(5), 214–222. https://doi.org/10.1159/000484899

Poma, H. R., Rajal, V. B., Blanco Fernandez, M. D., Barril, P. A., Giordano, M. O., Masachessi, G., et al. (2013). Evaluation of concentration efficiency of the *Pseudomonas aeruginosa* phage PP7 in various water matrixes by different methods. *Environmental Monitoring and Assessment, 185*(3), 2565–2576. https://doi.org/10.1007/s10661-012-2731-9

Selvaraju, S. B., Nix, W. A., Oberste, M. S., & Selvarangan, R. (2013). Optimization of a combined human parechovirus-enterovirus real-time reverse transcription-PCR assay and evaluation of a new parechovirus 3-specific assay for cerebrospinal fluid specimen testing. *Journal of Clinical Microbiology, 51*(2), 452–458. https://doi.org/10.1128/JCM.01982-12

Siqueira, J. D., Dominguez-Bello, M. G., Contreras, M., Lander, O., Caballero-Arias, H., Xutao, D., et al. (2018). Complex virome in feces from Amerindian children in isolated Amazonian villages. *Nature Communications, 9*(1), 4270. https://doi.org/10.1038/s41467-018-06502-9

Stocker, A., Souza, B. F., Ribeiro, T. C., Netto, E. M., Araujo, L. O., Correa, J. I., et al. (2012). Cosavirus infection in persons with and without gastroenteritis, Brazil. *Emerging Infectious Diseases, 18*(4), 656–659. https://doi.org/10.3201/eid1804.111415

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution, 30*(12), 2725–2729. https://doi.org/10.1093/molbev/mst197

Tan, S. Z., Tan, M. Z., & Prabakaran, M. (2017). Saffold virus, an emerging human cardiovirus. *Reviews in Medical Virology, 27*(1), e1908. https://doi.org/10.1002/rmv.1908

Texeira, P., Costa, S., Brown, B., Silva, S., Rodrigues, R., & Valério, E. (2020). Quantitative PCR detection of enteric viruses in wastewater and environmental water sources by the Lisbon municipality: A case study. *Water, 12*(2), 544.

Thongprachum, A., Fujimoto, T., Takanashi, S., Saito, H., Okitsu, S., Shimizu, H., et al. (2018). Detection of nineteen enteric viruses in raw sewage in Japan. *Infection, Genetics and Evolution, 63,* 17–23. https://doi.org/10.1016/j.meegid.2018.05.006

Uchii, K., Doi, H., Okahashi, T., Katano, I., Yamanaka, H., Sakata, M. K., et al. (2019). Comparison of inhibition resistance among PCR reagents for detection and quantification of environmental DNA. *Environmental DNA, 1,* 359–367. https://doi.org/10.1002/edn3.37

Yu, J. M., Ao, Y. Y., Li, L. L., & Duan, Z. J. (2017). Identification of a novel cosavirus species in faeces of children and its relationship with acute gastroenteritis in China. *Clinical Microbiology & Infection, 23*(8), 550–554. https://doi.org/10.1016/j.cmi.2017.02.018

Zell, R., Delwart, E., Gorbalenya, A. E., Hovi, T., King, A. M. Q., Knowles, N. J., et al. (2017). ICTV virus taxonomy profile:

Picornaviridae. *Journal of General Virology, 98*(10), 2421–2422. https://doi.org/10.1099/jgv.0.000911

Zhang, X. A., Lu, Q. B., Wo, Y., Zhao, J., Huang, D. D., Guo, C. T., et al. (2015). Prevalence and genetic characteristics of Saffold cardiovirus in China from 2009 to 2012. *Scientific Reports, 5,* 7704. https://doi.org/10.1038/srep07704