Analysis of the circulation of hepatitis A virus in Argentina since vaccine introduction

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Abstract

Hepatitis A virus (HAV) has shown intermediate endemicity in Argentina, but its incidence has decreased since vaccine introduction in 2005. Environmental surveillance was conducted in five rivers from Argentina from 2005 to 2012, complementing clinical information. HAV detection decreased since 2005, although its circulation continues, maintaining viral diversity but not undergoing antigenic drift. Most sequences belonged to subgenotype IA, closely related to Argentinean clinical sequences, but one belonged to proposed subgenotype IC, previously undetected in the country. Environmental surveillance might contribute to monitoring the single-dose vaccination schedule, representing not only strains causing disease but also the circulating population and the viral introductions.

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Hepatitis A virus (HAV), a member of the *Picornaviridae* family, is an important cause of acute hepatitis transmitted mainly through the faecal-oral route by contaminated food or water or by personal contacts. It has been classified into six genotypes (I–VI). In South America, only genotype I was reported and, particularly in Argentina, all published sequences belong to subgenotype IA [1–5], with a recent report of an IA–IB recombinant [6].

HAV infection has shown intermediate endemicity in Argentina and was the principal cause of liver transplants, with a rate of 6–12 cases of hepatitis every 100 000 inhabitants per year between 2000 and 2005. In 2005, a single-dose vaccine schedule in 12-month-old children was implemented all across the country. According to the Ministry of Health, the incidence of hepatitis by HAV decreased to < 1 case every 100 000 inhabitants per year and no liver transplants associated with HAV had been reported since 2007. Concomitantly, the age group with most HAV cases shifted from 5 to 9 years up to 2007 to 15 to 44 years in 2010 [7]. HAV may still circulate in Argentina by being released into the environment through wastewater, representing a threat to non-immune individuals.

The objective of this work was to conduct environmental surveillance of HAV released into the environment by wastewater discharges to evaluate its circulation in Argentina.

Rivers in the metropolitan district of Buenos Aires city and suburbs were sampled: Río de La Plata and Luján during 2005–2006 and 2009–2011 and Riachuelo during 2005–2006 and 2012. The Suquía River, in the central region around Córdoba city, and the Arias-Arenales River, in the northwest around Salta city, were sampled during 2009.

The Río de La Plata River receives the discharge of untreated sewage from the populated Buenos Aires city and its suburbs (7 790 000 inhabitants), while Riachuelo, Luján, Suquía and Arias-Arenales rivers receive sewage from different wastewater treatment plants involving primary or secondary treatments from significantly smaller populations (270 000–1 300 000). All the rivers receive undeclared sewage discharges, the Riachuelo River being one of the most contaminated rivers in Argentina. The study areas have been fully described in a previous work [8].

Water samples were concentrated as previously described [9]. Briefly, samples taken from rivers of Buenos Aires city and suburbs were concentrated using a method based on adsorption-elution to negatively charged membranes, samples from Suquía River were concentrated by high-speed centrifugation and polyethylene glycol precipitation and samples from Arias Arenales River were concentrated by tangential ultrafiltration. The HAV genome was detected by an RT-nested-PCR directed to the 5′UTR region (described in Data S1).

HAV was detected in all analysed rivers, but in different periods (Table 1).

During 2005–2006, the rivers around Buenos Aires city were sampled. The high contamination and the low flow (5.4 m^3/s) of Riachuelo River contributed to the frequent detection of HAV in this period. On the other hand, the high depuration capacity of the Río de la Plata River, with an average flow of 22 000 m^3/s, hindered a more frequent viral detection.

In different sampling periods during 2009–2012, 4–7 years after the beginning of vaccination, the presence of the viral genome was evaluated in rivers from three geographical areas (Table 1). In these areas the vaccination coverage among children under 1 year of age in mid-2011 was: 55.8% in Buenos Aires city and 81.5% in the province of Buenos Aires; 52.2% in Córdoba province and 46.6% in Salta province.

A noticeable decrease in HAV detection (from 93.3% in 2005 to 2006 to 0.0% in 2012, $p < 0.0001$) was observed in the Riachuelo River, which could be partially explained by the environmental sanitation carried out on this river since 2008. However, the concomitant decrease in enterovirus and norovirus contamination was more moderate (from 96.8 to 50.0% for enterovirus and from 80.8 to 50.0% for norovirus, data not shown). Changes in viral detection frequencies between different periods in Río de la Plata and Luján Rivers were not statistically significant, probably due to the low level of detection

($p = 0.56$ and $p = 0.19$, respectively). The Suquía and Arias-Arenales Rivers showed the presence of HAV during 2009, indicating the viral circulation until that date.

For further analysis, a 409 pb region of VP1-2A junction [3] of 23 out of the 43 positive samples was sequenced and a maximum likelihood phylogenetic tree with bootstrap support (1000 replicates) was built using PhyML v3.0[10] and jModeltest v0.1.1 [11]. All sequences belonged to genotype I and 22 out of the 23 were grouped under subgenotype IA (Fig. 1a). The remaining sequence was grouped with Spanish strains associated with the consumption of shellfish imported from Peru and described as a proposed subgenotype IC [12], along with sequences from Cameroon [13]. Recombination events in the VP1-2A region of this subgenotype were ruled out by using Simplot v3.5.1 [14].

Environmental sequences from subgenotype IA were distributed in three groups across the phylogenetic tree and were intermingled with sequences from clinical samples from Argentina. Two of these groups only consisted of South American sequences from Uruguay, Brazil, Venezuela and Argentina from 1997 to 2009. The remaining group also included sequences from Europe and Asia. Phylogeographic structure was confirmed using the BaTS v0.1 (Data S1) [15].

A substitution rate of 1.9×10^{-4} substitutions/site/year (s/s/y) (Highest Posterior Density 95% (HPD95%): 5.2×10^{-5}–3.4×10^{-4} s/s/y) estimated on the worldwide VP1-2A region of subgenotype IA sequences was used in the demographic reconstruction for the HAV IA Argentinean dataset. The uncorrelated lognormal molecular clock [16] and the Bayesian skyline plot coalescent models [17] implemented in BEAST v1.6.2 [18] were used. This analysis showed an increase in the effective number of infections (as a measure of the relative genetic diversity of the viral population) between 1995 and 2000, a slight decrease between 2000 and 2005, followed by a constant phase until 2010 (Fig. 1b). This demographic reconstruction of HAV IA genomes did not reflect the drastic fall in HAV cases that followed the introduction of the vaccine, observed in turn by other authors after the introduction of vaccination in France [19].

The analysis of differential selection pressures on individual VP1-2A amino acid sites between populations (before and after immunization) was evaluated by the method devel-

TABLE 1. River samples analysed for hepatitis A virus

Sampled river	Year	Number of samples	Positive samples (5′-UTR)	Detection frequency	Sequenced samples (VP1-2A junction)
Río de la Plata	2005	7	0	0/10 (0%)†	
	2006	3	0		
	2009	9	2	3/30 (10%)†	1
	2010	17	1		1
	2011	4	0		0
Luján	2005	5	0	1/7 (14.3%)‡	0
	2006	2	1		1
	2009	10	0	0/30 (0%)‡	0
	2010	16	0		0
	2011	4	0		0
Riachuelo	2005	16	15	28/30 (93.3%)*	7
	2006	14	13		10
	2012	12	0	0/12 (0%)*	0
Suquía	2009	28	4	4/28 (14.3%)	3
Arias-Arenales	2009	117	7	7/117 (6.0%)	0

p values from two-tailed Fisher's exact test: †$p = 0.56$; ‡$p = 0.19$; *$p < 0.0001$.

FIG. 1 (a) Maximum likelihood phylogenetic tree constructed with the VP1-2A region of HAV genotype I. Bootstrap values higher than 50% are shown for relevant groups. Subgenotype IB was used as outgroup. Sequences reported in this work are shown in bold and are available in the GenBank database under the accession numbers JX185617-JX185639. Locations from where environmental sequences were obtained are indicated as: RP, Río de La Plata River; MR, Riachuelo River; RS, Suquía River; WWTP, wastewater treatment plant that discharges in Suquía River; RL, Luján River. (b) Demographic reconstruction of HAV IA population in Argentina from 1940 to 2010 based on VP1-2A sequences from clinical and environmental samples. The lower and upper bounds of the Highest Posterior Density 95% (HPD95%) are indicated with thin blue lines.