

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

M. D. Blanco Fernández¹, C. Torres¹, G. Riviello-López², H. R. Poma³, V. B. Rajal^{3,4}, S. Nates⁵, D. M. Cisterna⁶, R. H. Campos¹ and V. A. Mbayed¹

1) Cátedra de Virología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires – CONICET, 2) Laboratorio Químico Prefectura Naval Argentina, Ciudad Autónoma de Buenos Aires, 3) INIQUI, Facultad de Ingeniería, Universidad Nacional de Salta – CONICET, Salta, Argentina, 4) Fogarty International Center, University of California at Davis, Davis, CA, USA, 5) Instituto de Virología 'Dr. J.M. Vanella', Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba and 6) Servicio de Neurovirosis, INEI -ANLIS 'Dr Carlos G. Malbrán', Ciudad Autónoma de Buenos Aires, Argentina

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.

Keywords: Argentina, environmental surveillance, genotypes, hepatitis A virus, immunization

Original Submission: 6 July 2012; **Revised Submission:** 31 August 2012; **Accepted:** 10 September 2012
Editor: G. Antonelli

Clin Microbiol Infect

Corresponding author: V. A. Mbayed, Cátedra de Virología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 4to piso, Ciudad Autónoma de Buenos Aires 1113, Argentina
E-mail: vmbayed@ffyb.uba.ar

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 Suquia 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, Suquia 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 Suquia 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).

	C	L	M	1	2	0	3	4	B	Dispatch: 28.9.12	Journal: CLM	CE: Gayathri J.	
										Author Received:	No. of pages: 4	PE: Gayathri	
	Journal Name									Manuscript No.			

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 m³/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 m³/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.

- 2 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

TABLE 1. River samples analysed for hepatitis A virus

Sampled river	Year	Number of samples	Positive samples (5'-UTR)	Detection frequency	Sequenced samples (VPI-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: [†] $p = 0.56$; [‡] $p = 0.19$; * $p < 0.0001$.

($p = 0.56$ 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.

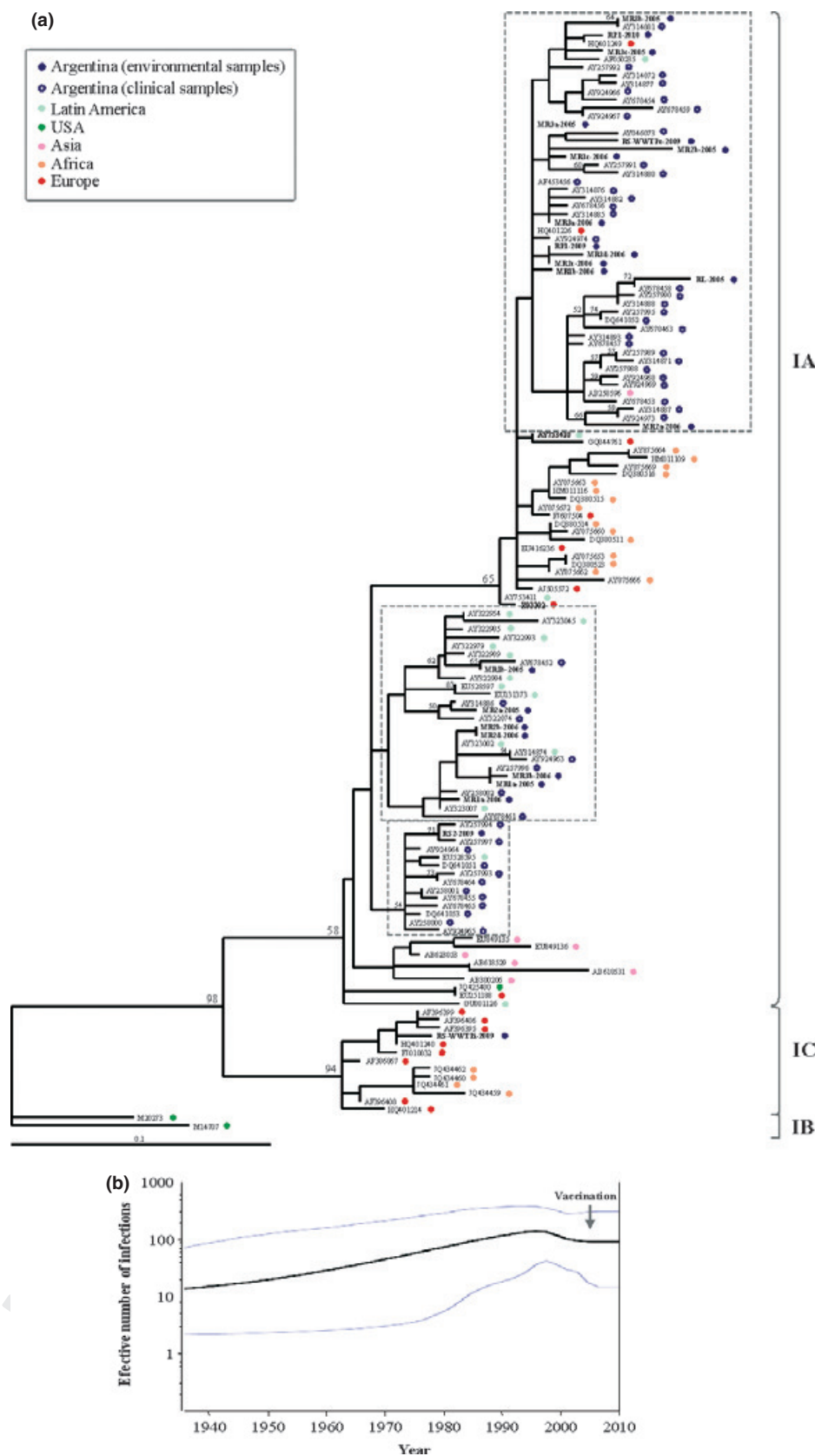
For further analysis, a 409 pb region of VPI-2A junction [3] of 23 out of the 43 positive samples was sequenced and a maximum likelihood phylogenetic tree with bootstrap support (1000 replicates) was built using PhyML v3.0 [10] and jModeltest v0.1.1 [11]. All sequences belonged to genotype I and 22 out of the 23 were grouped under subgenotype IA [3] (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 VPI-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 VPI-2A region [4] 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 VPI-2A amino acid sites between populations (before and after immunization) was evaluated by the method devel-

FIG. 1 (a) Maximum likelihood phylogenetic tree constructed with the VPI-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; VVWTP, 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 VPI-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.



oped by Pond *et al.* [20]. It showed that the viral population of HAV IA in Argentina did not appear to have undergone antigenic drift since the introduction of vaccination.

Summarizing, this study demonstrated the circulation of HAV in different regions of the country until 2009–2010. In 2012 a reduced detection of HAV genomes was observed in the Riachuelo River. The large decrease in the infection incidence due to vaccination [7] could have contributed to this. However, this decrease was not reflected by the demographic reconstruction of HAV genomes. Although this could be due to methodological shortcomings, it might indicate that viral circulation persists, mainly by non-notified asymptomatic infections, maintaining viral diversity. Environmental analysis might contribute to monitor the immunization approach implemented in Argentina and reviewed in 2012 by the Strategic Advisory Group of Experts on immunization of the WHO [21].

Finally, environmental surveillance has linked HAV detection in unknown individuals served by the wastewater system, to the viruses collected from clinical samples. HAV genomes from both sources were phylogenetically close, indicating that environmental surveillance might constitute a good representation of the viral population and provide valuable information, such as the introduction into Argentina of previously undetected subgenotype IC.

Transparency Declaration

XXXXX.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Detection of hepatitis A virus in environmental samples by nested-PCR of the 5' UTR.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

References

- Costa-Mattioli M, Cristina J, Romero H *et al.* Molecular evolution of hepatitis A virus: a new classification based on the complete vpI protein. *J Virol* 2002; 76: 9516–9525.
- Costa-Mattioli M, Ferre V, Monpoeho S *et al.* Genetic variability of hepatitis A virus in South America reveals heterogeneity and co-circulation during epidemic outbreaks. *J Gen Virol* 2001; 82: 2647–2652.
- Mbayed VA, Sookoian S, Alfonso V, Campos RH. Genetic characterization of hepatitis A virus isolates from Buenos Aires, Argentina. *J Med Virol* 2002; 68: 168–174.
- Munne MS, Vladimirovsky S, Moreira R *et al.* Molecular characterization of hepatitis A virus in children with fulminant hepatic failure in Argentina. *Liver Int* 2008; 28: 47–53.
- Munne MS, Vladimirovsky S, Otegui L *et al.* Molecular characterization of hepatitis A virus isolates from Argentina. *J Med Virol* 2007; 79: 887–894.
- Aguirre S, Malirat V, Scodeller E, Mattion N. First full-length genomic sequence of a hepatitis A virus isolated in Argentina shows recombination between subgenotypes IA and IB. *Virus Res* 2011; 155: 316–324.
- Ministerio de Salud. Presidencia de la Nación Argentina. *Boletín integrado de vigilancia* 2011; 95: 7–16.
- Blanco Fernández MD, Torres C, Poma HR *et al.* Environmental surveillance of norovirus in Argentina revealed distinct viral diversity patterns, seasonality and spatio-temporal diffusion processes. *Sci Total Environ* 2012; ????: ????. In Press.
- Poma HR, Rajal VB, Blanco Fernández MD *et al.* Evaluation of the virus concentration efficiency in water by different procedures using *Pseudomonas aeruginosa* phage PP7 as a viral model. *Environ Monit Assess* 2012; ????: ????. In Press.
- Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 2003; 52: 696–704.
- Posada D. JModelTest: phylogenetic model averaging. *Mol Biol Evol* 2008; 25: 1253–1256.
- Perez-Sautu U, Costafreda MI, Lite J *et al.* Molecular epidemiology of hepatitis A virus infections in Catalonia, Spain, 2005–2009: circulation of newly emerging strains. *J Clin Virol* 2011; 52: 98–102.
- Forbi JC, Agwale SM, Ndip LM, Esona MD. Genetic analysis of hepatitis A virus variants circulating among children presenting with acute diarrhea in Cameroon. *J Med Virol* 2012; 84: 728–732.
- Lole KS, Bollinger RC, Paranjape RS *et al.* Full-length human immunodeficiency virus type I genomes from subtype c-infected seroconverters in India, with evidence of intersubtype recombination. *J Virol* 1999; 73: 152–160.
- Parker J, Rambaut A, Pybus OG. Correlating viral phenotypes with phylogeny: accounting for phylogenetic uncertainty. *Infect Genet Evol* 2008; 8: 239–246.
- Drummond AJ, Ho SY, Phillips MJ, Rambaut A. Relaxed phylogenetics and dating with confidence. *PLoS Biol* 2006; 4: e88.
- Drummond AJ, Rambaut A, Shapiro B, Pybus OG. Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol Biol Evol* 2005; 22: 1185–1192.
- Drummond AJ, Rambaut A. Beast: bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 2007; 7: 214.
- Moratorio G, Costa-Mattioli M, Piovani R, Romero H, Musto H, Cristina J. Bayesian coalescent inference of hepatitis A virus populations: evolutionary rates and patterns. *J Gen Virol* 2007; 88: 3039–3042.
- Pond SL, Frost SD, Grossman Z, Gravenor MB, Richman DD, Brown AJ. Adaptation to different human populations by HIV-1 revealed by codon-based analyses. *PLoS Comput Biol* 2006; 2: e62.
- WHO. Meeting of the strategic advisory group of experts on immunization, April 2012 – conclusions and recommendations. *Wkly Epidemiol Rec* 2012; 21: 201–216.

Author Query Form

Journal: CLM

Article: I2034

Dear Author,

During the copy-editing of your paper, the following queries arose. Please respond to these by marking up your proofs with the necessary changes/additions. Please write your answers on the query sheet if there is insufficient space on the page proofs. Please write clearly and follow the conventions shown on the attached corrections sheet. If returning the proof by fax do not write too close to the paper's edge. Please remember that illegible mark-ups may delay publication.

Many thanks for your assistance.

Query reference	Query	Remarks
Q1	AUTHOR: Please check that authors and their affiliations are correct.	
Q2	AUTHOR: Does this read correctly now? 'A noticeable decrease in HAV detection...'	
Q3	AUTHOR: Does this read correctly now? '22 out of the 23 were grouped under' 'The remaining sequence was grouped with Spanish strains'	
Q4	AUTHOR: Does this read correctly now? 'estimated on the worldwide VPI-2A region'	
Q5	AUTHOR: Does this read correctly now? 'non-notified asymptomatic infections'	
Q6	AUTHOR: Does this read correctly now? 'detection in unknown individuals'	
Q7	AUTHOR: Please provide the 'Transparency Declaration' statement.	
Q8	AUTHOR: Please check the journal title for reference [7].	
Q9	AUTHOR: Please provide the volume number, page range for references [8 and 9].	

Q10	AUTHOR: Figure 1 has been saved at a low resolution of 205 dpi. Please resupply at 600 dpi. Check required artwork specifications at http://authorservices.wiley.com/submit_illust.asp?site=1	
-----	---	--

MARKED PROOF

Please correct and return this set

Please use the proof correction marks shown below for all alterations and corrections. If you wish to return your proof by fax you should ensure that all amendments are written clearly in dark ink and are made well within the page margins.

<i>Instruction to printer</i>	<i>Textual mark</i>	<i>Marginal mark</i>
Leave unchanged	... under matter to remain	Ⓟ
Insert in text the matter indicated in the margin	⧵	New matter followed by ⧵ or ⧵ [Ⓢ]
Delete	/ through single character, rule or underline or ⌵ through all characters to be deleted	⧶ or ⧶ [Ⓢ]
Substitute character or substitute part of one or more word(s)	/ through letter or ⌵ through characters	new character / or new characters /
Change to italics	— under matter to be changed	↵
Change to capitals	≡ under matter to be changed	≡
Change to small capitals	≡ under matter to be changed	≡
Change to bold type	~ under matter to be changed	~
Change to bold italic	≈ under matter to be changed	≈
Change to lower case	Encircle matter to be changed	≡
Change italic to upright type	(As above)	⧵
Change bold to non-bold type	(As above)	⧵
Insert 'superior' character	/ through character or ⧵ where required	Y or Y under character e.g. Y or Y
Insert 'inferior' character	(As above)	⧵ over character e.g. ⧵
Insert full stop	(As above)	⊙
Insert comma	(As above)	,
Insert single quotation marks	(As above)	Y or Y and/or Y or Y
Insert double quotation marks	(As above)	Y or Y and/or Y or Y
Insert hyphen	(As above)	⌵
Start new paragraph	⌵	⌵
No new paragraph	⌵	⌵
Transpose	⌵	⌵
Close up	linking ○ characters	○
Insert or substitute space between characters or words	/ through character or ⧵ where required	Y
Reduce space between characters or words		↑