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Evidence of Hepatitis A virus circulation in central Argentina: Seroprevalence and environmental surveillance

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ABSTRACT

Background: Hepatitis A virus (HAV) has shown intermediate endemicity in Argentina, but notification of clinical cases has decreased since the introduction of the vaccine in 2005.
Objectives: In order to get insight into the local circulation of this virus after four years of the official introduction of the vaccine, the aims of this study were to provide information on HAV immune status of the adult population of Córdoba city and to conduct environmental surveillance of HAV in sewage and river samples in the same region.
Study design: The prevalence of anti-HAV was determined by EIA in 416 samples of people (without prior vaccination) from Córdoba city (2009–2010). Spline regression models were estimated under generalized additive models. Environmental surveillance was conducted in river and sewage samples collected in the same period. Viral detection was performed by RT-Nested PCR of the 5′UTR.
Results: In Córdoba, the global prevalence of anti-HAV was 73.5%. It increased with age ($p < 0.0001$) and it was associated with the low-income population (OR: 1.14; 95% CI 1.05–1.25). This prevalence decreased in younger age groups, especially in the high-income population. Environmental monitoring revealed the presence of HAV (IA) in 20.8% and 16.1% of wastewater and river samples, respectively.
Conclusions: As a consequence of a decrease in HAV circulation due to improvements in immunization, socio-economic and hygienic conditions, young adults are becoming increasingly susceptible to HAV infections. Environmental monitoring demonstrated that HAV circulates in the local population; therefore, health care systems should consider the implementation of preventive measures for susceptible adults in order to reduce the risk of HAV infection.

1. Background

Infection with Hepatitis A virus (HAV) is the most common cause of acute viral hepatitis worldwide [1].

HAV has only one serotype which is organized into six genotypes (I–VI) [2]. In South America, only genotype I was found and, in Argentina, most of the reported sequences belong to subgenotype IA [3–7], with a recent report of an IA–IB recombinant [8] and HAV IC [9].

The most common vehicles for HAV transmission are: the ingestion of contaminated water, consumption of contaminated food and contact with infected people. HAV is very stable in the environment [10] and has shown to survive in diverse environmental and substrate conditions [11,12].

The incidence of HAV infection and the prevalence of anti-HAV are closely associated with economic development and access to safe potable water and sanitation. Low income, low educational level, overcrowding and lack of access to safe potable water and sanitary facilities are associated with an increase in the number of HAV infections [1].

In the past, Argentina was considered to be an area of high endemicity for HAV infection, with most people infected in early

Abbreviations: HAV, Hepatitis A virus; anti-HAV, HAV antibodies; EIA, enzyme immunoassay; IgM, immunoglobulin M; IgG, immunoglobulin G; OD, odd ratio; SP, sampling points; RT-Nested PCR, reverse transcription nested polymerase chain reaction; UTR, untranslated region; VP1-2A, variable protein1–2A; ARG-CBA-WW, Argentina–Córdoba–Wastewater.

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childhood [13]. Then, HAV was the principal cause of liver transplant, with a rate of reported cases of hepatitis ranging from 70.5 to 173.8 every 100,000 inhabitants per year during 1995–2004 [14]. In 2005, vaccination against HAV was included in the national immunization program in a one-dose scheme among children aged 12 months [15]. After initiating this program, the annual incidence of 10.2 per 100,000 inhabitants during 2007 represented a 88.0% reduction with respect to the average incidence rate for the period 1998–2002. Concomitantly, the age group with most HAV notified cases shifted from children (5–9 years) until 2007 to young adults (15–44 years) in 2010 [16]. Previous seroprevalence studies performed in the metropolitan region of Argentina (Buenos Aires) showed that over 50% of young people less than 30 years old were susceptible to HAV. It also showed that the lower prevalence rates were found in middle and high socioeconomic and health conditions [13,17]. In Córdoba (the second most populated inland province of Argentina), recent information about HAV circulation and seroprevalence data in the adult population are not available.

The virus detection in urban recreational water is very important to identify viral infectious sources. Since HAV infections have been linked to outbreaks of waterborne diseases [18], the environmental surveillance through the study of sewage can provide valuable supplementary information. This is particularly important in urban populations with absent or questionable surveillance, when persistent virus circulation is suspected, or frequent virus re-introduction occurs [19]. Although the universal vaccination strategy has provided cost-effective results, molecular epidemiological surveillance of circulating viruses is essential to identify changes with this strategy. In this sense, the bibliography has been enriched lately by several studies which have demonstrated the advantage of environmental surveillance as an additional tool to determine the epidemiology of different viruses circulating in a given community [20,21].

2. Objectives

The purpose of this study was to provide information of HAV circulation in Córdoba, central region of Argentina, by seroprevalence analysis and environmental surveillance (raw sewage and watercourses of urbanized river) four years after HAV vaccination was introduced.

3. Study design

3.1. Serum samples

A retrospective study was carried out in Córdoba City in 416 serum samples from individuals with no record of previous vaccination, and who attended health care centers during September 2009 and September 2010. The individuals enrolled were classified into 5 groups according to age (15–78 years old: 15–25 years old, 26–35 years old, 36–45 years old, 46–55 years old and older than 56 years old) and two groups according to socioeconomic level (low-income population and middle/high-income populations) following a classification provided by the Municipality of Córdoba, which is based on the economic, social and educational level of each individual [22]. The location of collected samples is shown in Fig. 1A.

This work is part of a research project registered and approved by the Ethics Committee of the Health Ministry of the Province of Córdoba.

3.2. Serological test

Anti-HAV was detected using a commercial competitive enzyme immunoassay kit (EIA, Diapro, Milan, Italy). This qualitative assay determined the presence of total anti-HAV (IgM and IgG). Ratio of cut-off to sample OD greater than 1.1 was considered positive.

3.3. Statistical analysis

Spline regression models were estimated in the context of generalized additive models [23,24]. In order to assess social categories, once the estimates of the mean curves were obtained, a comparison of these curves (along its entire length or in sections) were analyzed using a hypothesis test estimated by bootstrap [25]. The estimates were obtained using maximum likelihood controlled by the parameter λ [26]. Risk factors associated with HAV infection and socio-demographic characteristics were identified by using multiple logistic regression models.

3.4. Raw sewage treatment plant and river samples collection

Samples from sewage (n = 24) were periodically collected during a two-year period (2009–2010). Coverage of the sewerage network is about 61% of the population (1,330,023 inhabitants, census 2010) and it is located in southeast of Córdoba city (Fig. 1B).

The wastewater treatment process involves mechanical methods, such as retention of solid materials by grids and then removal of particulate matter by sedimentation and flocculation, with a final treatment process in chlorine basins.

Samples (n = 31) of the Suquia River were collected seasonally during 2010 in eight sampling points (SP) that cover a whole of its course across the town (Fig. 1B). During spring, the water sample from point 7 could not be collected.

For each sample, 1500 ml of sewage was collected in sterile plastic bottles, stored at 4 °C and transported to the laboratory for immediate analysis.

3.5. Sample concentration, viral extraction and molecular analysis

Samples were concentrated 100× by centrifugation and polyethylenglicol precipitation following the protocol described by Lewis and Metcalf [27]. RNA was extracted from 140 μl of concentrated samples using a QIAamp® Viral RNA Kit (Qiagen GmbH, Germany). RT-Nested PCR techniques have been used for the detection of a 230 pb fragment of the 5′UTR region and 409 pb of VP1-2A junction of the genome of HAV [5,9]. PCR products were purified using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, and USA) and sequenced in both directions by an ABI automatic sequencer. Nucleotide sequences were analyzed by BLAST 2.2.19 [28] to determine the identity of the amplicons. A dendrogram was constructed with the program Mega version 4.0 software (Center for Evolutionary Functional Genomics, Arizona, USA) using the neighbor joining method and p-distance parameter [29].

4. Results

4.1. Serological test

Anti-HAV was found in 73.5% (306/416) of serum samples. According to the socioeconomic level of the studied population, the total prevalence of anti-HAV in low-income populations was 81.9% (159/194) and 66.2% (147/222) in middle/high-income populations. The percentages of anti-HAV in each age group and socioeconomic characteristics are shown in Table 1.

The prevalence of anti-HAV increased with age and with different trends by socio-economic variables (p = 0.034). Throughout age groups, these differences were stressed, showing homogeneity only from age 45 (p = 0.473). So, anti-HAV was markedly higher

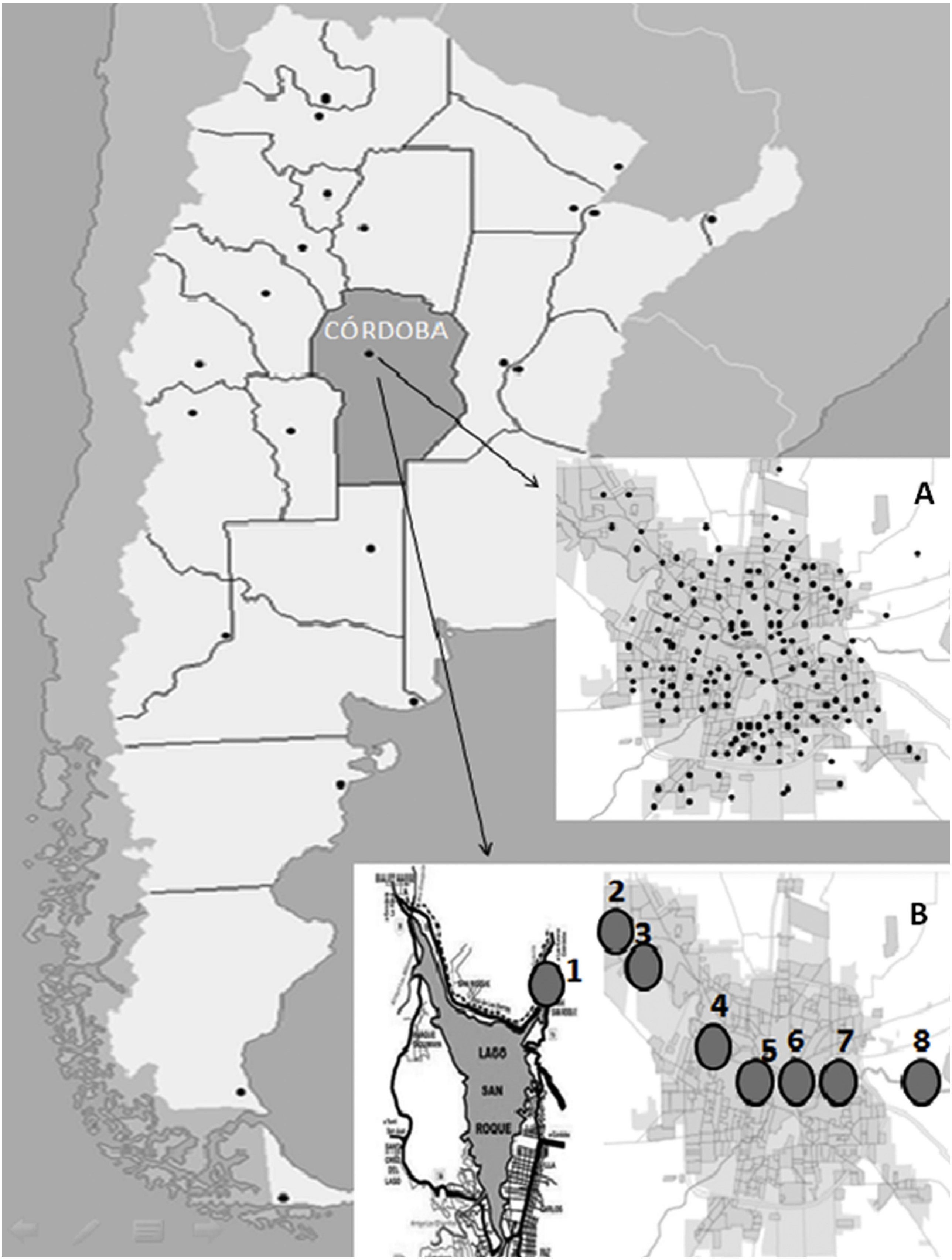

Fig. 1. Map showing location of the city of Córdoba, in the province of Córdoba, Argentina. (A) Map of Cordoba city showing approximate location of the samples collected for serological study and (B) location of the sampling points throughout the Suquia River. Sewage treatment plant is located between sampling points 7 and 8.

Table 1
Percentage of individuals within each age group and socioeconomics characteristics with anti-HAV serum antibodies.

Age (*y*)	N anti-HAV+, %(*n*)	Middle/high income population anti-HAV+, %(*n*)	Low income population anti-HAV+, %(*n*)	*p* Value
15–25	53.2 (50/94)	41.5 (17/41)	62.3 (33/53)	0.05
26–35	59.2 (64/108)	46.8 (29/62)	76.1 (35/46)	<0.001
36–45	77.1 (54/70)	67.5 (27/40)	90.0 (27/30)	<0.001
46–55	92.7 (64/69)	89.8 (35/39)	96.7 (29/30)	NS*
>56	98.7 (74/75)	97.5 (39/40)	100 (35/35)	NS*
Total	73.5 (306/416)	66.2 (147/222)	81.9 (159/194)	0.034

* A p value > 0.05 was considered non-statistically significant.

between age 26 and 45 in the low-income populations (76–90%) than in middle/high-income populations (47–67%) ($p < 0.001$).

Overall, and across all age groups, there was approximately 24% higher prevalence of anti-HAV in the low-income population compared to the middle/high-income population ($p < 0.05$).

4.2. Environmental surveillance

Environmental monitoring revealed the presence of HAV in 20.8% (5/24) and 16.1% (5/31) of the raw sewage samples and river samples analyzed, respectively.

HAV was found intermittently in both sewage water and rivers, so a seasonal pattern could not be confirmed. It was found in sewage samples in spring 2009 (October and November), summer 2010 (January and February) and winter 2010 (June). In surface river samples (sampling 2010), HAV was detected in SP1 (summer and winter), SP3 (summer), SP5 (summer) and SP6 (winter) (Fig. 1B). No amplification was obtained for any samples in the VP1-2A genomic region.

Genetic sequencing of the 5′UTR obtained from three raw sewage samples allowed to assign HAV strains as genotype I, subgenotyping IA, revealing 97–98% homology compared to subgenotype IA from HAV GBM wild-type IA sequence (see Supplementary Fig. 2). The partial genomic sequence of the isolates named ARG-CBA-WW Oct09, Nov09 and Jun10 has been deposited in the GenBank database under the accession numbers KF300769–KF300771.

5. Discussion

HAV infection used to be a benign childhood illness. Now, as both sanitary and socioeconomic conditions have improved in some areas of the world, the age of infection has shifted to older groups, and thus the number of symptomatic cases has increased. The seriousness of the disease is related to the age of the subject involved [30].

Since the introduction of the HAV vaccine in 2005, Argentina would not be far from this scene, but not data is available. As expected, this study found that seroprevalence of anti-HAV increased with age and was influenced by the socioeconomic status. There was 24% higher prevalence in individuals belonging to low-income populations compared with individuals belonging to middle/high-income populations. In the majority of the cases studied (~90%), the people involved lived in houses with tap water. However, some people lived in suburban areas where the potable water supply was not available. The possibility of water contamination with sewage potentially containing HAV cannot be excluded.

As regard changes in prevalence by age groups, we found that the prevalence of antibodies found in the studied population was high (73%), and increased with age to more than 90% in adults over fifty. Individuals belonging to low-income populations had higher total seroprevalence (81.9%) than individuals belonging to middle/high-income populations (66.2%). This seroprevalence also increases with age. In low-income populations, the prevalence of anti-HAV was higher than 60% in young individual (>15 years); however, this seroprevalence was lower than 50% between subjects belonging to middle/high-income populations of the same age group, rising to 60% in young adults (35–46 years). This increase in prevalence with age could be explained either by the years of exposure to the virus or the improved sanitary conditions that now exist compared to those in previous decades. The high percentage of seroprevalence in people older than 50 years old may reflect the lower standards of hygiene common in the first half of the century. Previous studies in Argentina [13] had shown 70–80% of seroprevalence among individuals between 21 and 40 years old. These results are similar to those found in the present study but only in individuals of low socioeconomic conditions. According to the results, the prevalence of anti-HAV exhibits a pattern of intermediate endemicity in Córdoba. However, based on the following definitions previously published [13] we could reconsider some issues: (a) In high endemicity areas, the incidence varies from low to high, with a peak age of infection in early childhood. The transmission pattern is from person to person and outbreaks are uncommon. (b) In moderate endemicity areas, the incidence is high and the peak age of infection is in the late childhood/adolescence or in young adults. The transmission pattern is also from person to person, and by food and water. Outbreaks are common. (c) In low endemicity areas, the incidence is low, with the peak age of infection in young adulthood. The transmission pattern is from person to person, and also by food and water. Outbreaks are common. Since the inclusion of the HAV vaccine in the routine childhood immunization schedule of Argentina (in the year 2005) with a coverage of 80–90%, the incidence of cases in our region is very low (in 2007: 7.8/100,000 inhabitants) [14]. Also, prevalence rates were found less than 50% specifically in high-income young adults and the peak age of infection was in young adulthood. In connection with the previous information, the endemicity of HAV, at least in the middle/high-income group, would be changing its behavior exhibiting a pattern of low endemicity.

In this context, it is essential to continue with the official policy of vaccination, but we should adjust epidemiological surveillance and catch-up vaccination for adolescents and young adults.

Environmental monitoring helped to demonstrate that HAV circulated in sewage and river samples during the same period in which susceptible individuals were found, which indicated a risk for these individuals.

Worldwide, HAV detection in environmental and potable water samples is becoming an important strategy for preventing outbreaks of infection with waterborne viruses. Water quality, sanitation and health are closely interrelated. Suquia River flows through the entire landscape of Córdoba city (37.6 km). This watercourse is widely used by a large number of inhabitants of the city for recreational activities, and it could be a source of transmission of enteric viruses. Previous studies in the same river demonstrated the presence of norovirus [31], and in the year 2009 the presence of HAV subgenotype IA and IC was reported in 14.3%

of the samples analyzed [9]. This detection rate was similar to that found in the present study during the year 2010 (16.1%) and had no particular seasonal pattern. The frequency of HAV detection in the Suquia River could be influenced not only by the circulation of the virus in the human population, but also by the characteristics of the river, as the flow and treatment capacity.

Wastewater has been described as an important source for spreading pathogenic microorganisms in the environment. However, in our region there are few studies that report the presence of gastroenteric viruses in these environmental matrices [32,33].

The results of the present study demonstrated the presence of HAV in raw sewage samples obtained from the municipal wastewater treatment plant of Córdoba city.

The frequency of HAV detection in this source was variable and was found intermittently, without a seasonal pattern. However, HAV was found in the same seasons in both sewage and river samples in the year 2010.

In wastewater samples which tested positive for HAV, only genotype I, subgenotype IA was found. However, a limitation of our study was the impossibility to obtain sequences of the VP1-2A region. The failure of amplification may be due to a sensitivity problem as a result of the low number of viral particles present in water samples. This was evidenced in other reports in which the detection efficiency of the PCR to amplify VP region was much lower than the PCR for 5′UTR amplification [9]. Even though VP1-2 region has been extensively studied to infer phylogenetic relationships among HAV genotypes and subgenotype [4,5], genetic characterization by analyzing 5′UTR has previously been reported [34–36]. Although the amplified fragment corresponding to the 5′UTR gene was short and not highly variable to perform detailed phylogenetic analyses, its analysis provided evidence of the HAV subgenotype IA presence in environmental samples from Córdoba. Coincidentally, this subgenotype was also predominant in clinical samples collected from Argentinian patients [5,6].

The presence of HAV in local environmental samples and the significant decline in immunity in young adults raises a favorable scenario for the production of outbreaks. This finding has been described in parts of Central and Eastern Europe [30]. These outbreaks have been linked to transmission due to poor living conditions (shared facilities), food handling by contaminated kitchen staff, and the return of inmigrants' children from visits to their countries of origin with higher endemicity, and carers of children. In the same way, two outbreaks were described in Prague, Czech Republic, in the second half of 2008 (the first major outbreak there for 12 years which particularly affected young adults and adults) and in adults from Latvia during autumn 2007 (after eight years with less than 100 cases reported a year) [30].

Persistent environmental monitoring and the implementation of preventative measures must be considered in order to deal with the risk related to this epidemiological shift in Argentina.

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Competing interests

None declared.

Authors' contributions

VER, MBI and SVN were involved in the study design, the analysis of the data and in the process of writing the manuscript.

LAY, NSL, MMT and LIS collected data and carried out the experiments. LAY and PAB were involved in the analysis of data and in the process of writing the manuscript. MPD performed statistical analysis. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2013.11.005.

References

[1] Jacobsen KH, Wiersma ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. Vaccine 2010;28:6653–7.
[2] Robertson BH, Jansen RW, Khanna B, Totsuka A, Nainan OV, Siegl G, et al. Genetic relatedness of hepatitis A virus strains recovered from different geographical regions. J Gen Virol 1992;73:1365–77.
[3] Costa-Mattioli M, Ferre V, Monpoeho S, Garcia L, Colina R, Billaudel S, et al. Genetic variability of hepatitis A virus in South America reveals heterogeneity and co-circulation during epidemic outbreaks. J Gen Virol 2001;82:2647–52.
[4] Costa-Mattioli M, Cristina J, Romero H, Perez-Bercof R, Casane D, Colina R, et al. Molecular evolution of hepatitis A virus: a new classification based on the complete VP1 protein. J Gen Virol 2002;76:9516–25.
[5] Mbayed VA, Sookoian S, Alfonso V, Campos RH. Genetic characterization of hepatitis A virus isolates from Buenos Aires, Argentina. J Med Virol 2002;68:168–74.
[6] Munné MS, Vladimirsky S, Otegui L, Soto S, Brajterman L, Castro R, et al. Molecular characterization of hepatitis A virus isolates from Argentina. J Med Virol 2007;79:887–94.
[7] Munné MS, Vladimirsky S, Moreiro R, Ciocca M, Cuarterolo M, Otegui L, et al. Molecular characterization of hepatitis A virus in children with fulminant hepatic failure in Argentina. Liver Int 2008;28:47–53.
[8] 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;155:316–24.
[9] Blanco Fernández MD, Torres C, Riviello-López G, Poma HR, Rajal VB, Nates SV, et al. Analysis of the circulation of hepatitis A virus in Argentina since vaccine introduction. Clin Microbiol Infect 2012;18:548–51.
[10] Sosbey M, Shields PA, Hauchman FS, Davis AL, Rullman BA, Bosh A. Survival and persistence of hepatitis A virus in environmental samples. In: Zuckerman AJ, editor. Viral hepatitis and liver disease. New York: A.R. Liss; 1988. p. 121–4.
[11] Cuthbert JA. Hepatitis A: old and new. Clin Microbiol Rev 2001;14:38–58.
[12] Morace G, Aulicino F, Angelozzi C, Costanzo L, Donadio F, Rapicatta M. Microbial quality of wastewater: detection of hepatitis A virus by reverse transcriptase-polymerase chain reaction. J Appl Microbiol 2002;92:823–36.
[13] Tanaka J. Hepatitis A shifting epidemiology in Latin America. Vaccine 2000;18:57–60.
[14] Vacchino MN. Incidence of Hepatitis A in Argentina after vaccination. J Viral Hepat 2008;15:47–50.
[15] Ministry of Health of Argentina. National Immunization Guidelines [http://www.msal.gov.ar/images/stories/epidemiologia/pdf/normas-vacunacion-08.pdf].
[16] Infectious Diseases Society of Argentina. Update of Immunization Recommendations [http://www.sadi.org.ar/files/Actualizacionenvacunas_SADI2008.pdf].
[17] Lopez H, Zitto T, Bare P, Vidal G, Vukasovic J, Gomez R. Prevalence of anti-hepatitis A antibodies in an urban middle class area of Argentina: some associated factors. Int J Infect Dis 1999;4:34–7.
[18] Pinto R, Alegre D, Dominguez A, El-Senousy W, Sanchez G, Villena C, et al. Hepatitis A virus in urban sewage from two Mediterranean countries. Epidem Inf 2007;135:270–3.
[19] WHO. Guidelines for Environmental Surveillance of Poliovirus Circulation. Geneva: World Health Organization; 2003.
[20] Kokkinos P, Ziros P, Meri D, Filippidou S, Stella Kolla S, Galanis A, et al. Environmental surveillance. An additional/alternative approach for virological surveillance in Greece? Int J Environ Res Public Health 2011;8:1914–22.
[21] Prado T, Fumian TM, Miagostovich MP, Gaspar AM. Monitoring the hepatitis A virus in urban wastewater from Rio de Janeiro, Brazil. Trans R Soc Trop Med Hyg 2012;106:104–9.

[22] Aballay LR, Osella AR, Celi A, Díaz MP. Overweight and obesity: prevalence and their association with some social characteristics in a random sample population-based study in Córdoba city, Argentina. Obes Res Clin Pract 2009;3:75–83.
[23] Hastie T, Tibshirani R. Generalized additive models. London: Chapman and Hall; 1990.
[24] Rigby RA, Stasinopoulos DM. Smooth centile curves for skew and kur-totic data modelled using the Box–Cox Power Exponential distribution. Stat Med 2004;23:3053–76.
[25] Nores ML, Wunderlin DA, Díaz MP. Bootstrap hypothesis testing in generalized additive models for comparing curves of treatments in longitudinal studies. In: Proceedings of the twenty-fifth International Biometric Conference. 2010.
[26] Wood SN. Generalized additive models: an introduction with R. New York: Chapman and Hall; 2006.
[27] Lewis GD, Metcalf TG. Polyethylene glycol precipitation for recovery of pathogenic viruses, including hepatitis A viruses and human rotavirus, from oyster, water and sediment samples. Appl Environ Microbiol 1998;54: 1983–8.
[28] Zhang Z, Schwarts S, Wagner L, Miller W. A greedy algorithm for aligning DNA sequences. J Comput Biol 2000;7:203–14.
[29] Tamura K, Dudley J, Nei M, Kumar S. Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 2007;24:1596–9.
[30] FitzSimons D, Hendrickx G, Vorsters A, Van Damme P. Hepatitis A and E: update on prevention and epidemiology. Vaccine 2010;28:583–8.
[31] Blanco Fernández MD, Torres C, Martínez LC, Giordano MO, Masachessi G, Barril PA, et al. Genetic and evolutionary characterization of norovirus from sewage and surface water in Córdoba City, Argentina. Infect Genet Evol 2011;11:1631–7.
[32] Barril PA, Giordano MO, Isa MB, Masachessi G, Ferreyra LJ, Castello AA, et al. Correlation between rotavirus A genotypes detected in hospitalized children and sewage samples in 2006, Córdoba, Argentina. J Med Virol 2010;82:1277–81.
[33] Mueller JE, Bessaud M, Huang QS, Martinez LC, Barril PA, Morel V, et al. Environmental poliovirus surveillance during oral poliovirus vaccine and inactivated poliovirus vaccine use in Córdoba Province, Argentina. Appl Environ Microbiol 2009;75:1395–401.
[34] Villar LM, Morais LM, Aloise R, Melo MMM, Calado IA, Lampe E, et al. Co-circulation of genotypes IA and IB of hepatitis A virus in Northeast Brazil. Braz J Med Biol Res 2006;39:873–81.
[35] Davaalkham D, Enkhoyun T, Takahashi M, Nakamura Y, Okamoto H. Hepatitis A and E virus infections among children in Mongolia. Am J Trop Med Hyg 2009;81:248–51.
[36] Kanda T, Jeong S-H, Imazeki F, Fujiwara K, Yokosuka O. Analysis of 59 nontranslated region of hepatitis A viral RNA genotype I from South Korea: comparison with disease severities. PLoS One 2010;5:e15139.