Single-dose Universal Hepatitis A Immunization in One-year-old Children in Argentina

High Prevalence of Protective Antibodies up to 9 Years After Vaccination

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Background: Single-dose hepatitis A virus (HAV) vaccination was implemented in all Argentinean children 12 months of age in 2005. Previous studies demonstrated high prevalence of protective antibody response 4 years after single-dose vaccination. This study assessed long-term seroprotection against HAV after vaccination.

Methods: Children who received 1 dose of HAV vaccine at 1 year of age at least 6 years before enrollment were included at 5 centers in Argentina between 2013 and 2014. Demographic and socioeconomic characteristics were collected through a questionnaire. Blood samples were tested for anti-HAV antibodies. Antibody values ≥10 mIU/mL were considered seroprotective. Logistic regression analysis was performed to evaluate the association between demographic and socioeconomic variables and seroprotection.

Results: A total of 1088 children were included, with a median postvaccination interval of 7.7 years (range 6.3–9.2 years). Of these children, 97.4% (95% confidence interval: 96.3%–98.3%) had protective antibodies against HAV. No association between demographic or socioeconomic variables and seroprotection was found. Geometric mean concentration of antibody levels against HAV was 170.5 mUI/mL (95% confidence interval: 163.2–178.2 mUI/mL).

Conclusions: Single-dose universal hepatitis A immunization in 1-year-old children resulted in sustained immunologic protection for up to 9 years in Argentina. These findings, along with the low current disease burden, confirm the success of the intervention.

Key Words: hepatitis A, single-dose vaccination, children, immunogenicity, long-term follow-up, Argentina

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Vaccination against hepatitis A has been shown to be safe and effective, and resulted in sustained high levels of protection.[1–4] When implemented universally, it has also proved to rapidly decrease hepatitis A incidence rates and control the disease.[5–8] However, the high cost and need of a 2-dose schedule has limited its widespread use in most low- and middle-income countries.

Universal single-dose vaccination against hepatitis A was implemented in Argentina in June 2005 for all infants 12 months of age, after a nationwide outbreak in 2003 to 2004.[9,10] At that time, hepatitis A was the leading cause of fulminant hepatic failure and liver transplantation in children.[11–13] The single-dose vaccination strategy was decided at that moment based on (1) previous data demonstrating that 85%–95% of vaccinated individuals achieve protective antibody titers 10–14 days after the first dose, and almost 100% seroconvert after 4–6 weeks[1–4]; (2) the experience from other countries where transmission was interrupted even in older age groups as a result of vaccinating the younger cohorts (herd protection)[5–8]; (3) the expectation that in addition to the humoral response, immunological memory recall could be elicited after a single dose in children, as it was demonstrated in adults[14,15]; and finally, (4) the economic limitations in Argentina at that time to include a 2-dose series; so, if 1-dose was sufficient, it would make for an affordable and sustainable long-term vaccination strategy.

Concerns regarding effectiveness of this strategy, as well as whether a booster dose would be needed in the future, arose at that time. After immunization, it is expected that anti-HAV titers will decrease in the next several years.[16,17] Still, immunity waning is expected to be gradual, and it has been estimated that HAV vaccine–induced protective antibodies could persist for more than 20 years in adults, and up to 14 and 24.5 years in children from China and Taiwan, respectively.[16–18] However, these estimations were performed after a 2- or 3-dose schedule, and not only in a different epidemiological context to that of Argentina, but under their own sanitary conditions. Thus, the length of protection remained uncertain after a single-dose vaccination in our country.

A continuous and strengthened surveillance was implemented by the Ministry of Health of Argentina after the start of the intervention.[19] Between 2005 and 2011, a dramatic decline was observed in HAV infection rates, fulminant hepatitis and liver transplantation, which is sustained to current days.[20,21] Moreover, persistence of protective antibodies in 5-year-old children who received a single dose of hepatitis A vaccine at 12 months was assessed in 2011. In that study, more than 93% of that population maintained protective antibody levels up to 4 years after vaccination.[22]

Based on those outcomes, the World Health Organization Strategic Advisory Group of Experts on Immunization concluded, in April 2012, that national immunization programs may consider the inclusion of single-dose inactivated hepatitis A vaccines in immunization schedules as a good alternative to the standard 2-dose regimen and that long-term protection from 1- or 2-dose schedules should be regularly monitored by local health authorities.[23]

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In accordance with that recommendation, a second seroprevalence study was carried out by the Ministry of Health during 2013. This study attempted to assess long-term prevalence of protective antibodies in children after vaccination with single-dose hepatitis A vaccine.

MATERIALS AND METHODS

Same methodology as described in previous seroprevalence study was adopted in this trial.[22]

Study Population

Participants were enrolled from 16 March 2013 to 30 April 2014 from same centers as in the 2011 study, which belong to historically low, middle and high endemic hepatitis A regions of the country.[24] The following centers were selected: Hospital de Niños Ricardo Gutiérrez (Buenos Aires city), Hospital de Niños de San Justo and Hospital Nacional Prof. Dr. Alejandro Posadas (Buenos Aires province), Hospital de Niños de la Ciudad de Tucumán (Tucumán city) and Hospital de Niños Orlando Alassia (Santa Fe city). Buenos Aires city and both Buenos Aires and Santa Fe provinces are located in the central region of the country, whereas Tucumán is in the northwestern region of Argentina. Children who visited these health care centers for routine well-child visits were screened to participate in the study.

To estimate the prevalence of anti-HAV protective antibodies, children who had received a single dose of HAV vaccine at 12 +6/-1 months of age at least 6 years before entering the study were included. Children with a history of hepatitis A, primary or secondary immunodeficiency, chronic disease or any active illness at the time of enrollment, as well as those who were participating in other trials, or whose parents did not give written informed consent to participate in this study were excluded.

The study was approved by each hospital's ethics committee and by the Ministry of Health of Argentina.

Data Collection and Laboratory Assessments

Demographic and socioeconomic data were collected using a standardized questionnaire completed by the children's parents. Blood sample analysis was performed at the Hepatitis and Gastroenteritis Service, Virology Department, National Reference Laboratory for Viral Hepatitis at the National Institute of Infectious Diseases–ANLIS "Carlos Malbrán" in Buenos Aires city. Serum was tested for anti-HAV antibodies using the commercially available AxSYM HAVAB 2.0 microparticle enzyme immunoassay (Abbott Laboratories, Abbott Park, IL). Seropositivity was defined as antibody levels of ≥10 mIU/mL.[25,26] Children with antibody levels of <10 mIU/mL were offered to receive a second dose of vaccine as immediate response after single-dose priming vaccination was not assessed at that time to rely on immunological memory recall. No additional measurement of antibodies was performed thereafter.

Statistical Analysis

Descriptive statistics and seroprevalence results were obtained using Epi Info 7 program (Centers for Disease Control and Prevention, Atlanta, GA). Means and 95% confidence intervals (CIs) or medians and ranges were reported for continuous variables. Proportions were reported for the categorical variables. To evaluate the association between demographic and socioeconomic variables and antibody seropositivity, standard test was used for continuous variables, *t* test or Wilcoxon rank sum tests; and χ^2 tests for proportions for the categorical variables; *t*-adjusted odds ratios with their 95% CIs were also used. Multivariate analysis was performed for significant variables from the previous bivariate analysis using logistic regression analysis.

RESULTS

A total of 1133 children were recruited for this study. Of these, 45 children were excluded: 33 cases because children were vaccinated with >18 months; in 5 cases, date of vaccination could not be proven; in 1 case, the child was not vaccinated; and in 6 cases, samples were missed and could not be processed at the reference laboratory. Demographic characteristics of the remaining children and their mothers are shown in Table 1. Hospital de Niños de Tucumán in Tucumán city included more children than the other centers. However, the majority of the children (40.7%) lived in Buenos Aires province as 3 centers enrolled children from this region. There were a similar proportion of males and females, the great majority lived in urban areas and most had access to safe water. Almost half of the population studied had sewers, and the rest had cesspools or septic tanks for excreta disposal. Only few mothers had tertiary or university education levels. The children's median age was 8.7 (range: 7.33–10.5) years and median time after vaccination was 7.7 (range: 6.3–9.2) years, which were similar to the mean values.

Of the 1088 children that fulfilled inclusion criteria, and did not have exclusion criteria, 1060 (97.4%) (95% CI: 96.3%–98.3%) had anti-HAV antibody levels of ≥10 mIU/mL. Multivariate analysis showed no significant difference between children or mother's demographic or socioeconomic variables and the proportion of children with protective antibodies. Regarding age, age at vaccination and time postvaccination, the analysis again showed no significant differences between children with or without protective antibodies (Table 2).

Anti-HAV geometric mean concentration (GMC) was 170.5 mIU/mL (95% CI: 163.2–178.2 mIU/mL). Figure 1 shows the reverse cumulative distribution of antibody levels.

TABLE 1. Demographic and Socioeconomic Characteristics of the Study Group

Population Characteristics	n = 1088
Province	
Buenos Aires, n (%)	443 (40.7)
Tucumán, n (%)	351 (32.3)
Santa Fe, n (%)	258 (23.7)
Buenos Aires city, n (%)	34 (3.1)
Other, n (%)	2 (0.2)
Urban residence, n (%)	1035 (95.6)
Housing type	
House/department, n (%)	804 (74.2)
Other,* n (%)	284 (25.8)
Overcrowding,† n (%)	231 (21.2)
Number of people per room, mean (95% CI)	2.71 (2.64–2.78)
Access to tap water, n (%)	911 (83.9)
Attendance to school, n (%)	1085 (99,8)
Excretal disposal sewers, n (%)	517 (47.5)
Male sex, n (%)	522 (48.0)
Age (years), mean (95% CI)	8.75 (8.71–8.78)
Age at vaccination (months), mean (95% CI)	12.8 (12.7–12.8)
Time postvaccination (years), mean (95% CI)	7.7 (7.66–7.73)
Mother's educational level	
Primary/not completed secondary school, n (%)	700 (64.7)
Completed secondary school, n (%)	279 (25.8)
Tertiary/university, n (%)	103 (9.5)
Mother's nationality Argentine, n (%)	1024 (94.3)

*Includes type B houses, ranches/boxes, tenements, pensions/hotels, locals not built for room, trailers.

†Overcrowding was considered if there was ≥4 people per room.

TABLE 2. Multivariate Analysis of Main Selected Variables

Population Characteristics	Protective Antibody Titers (Anti-HAV ≥10 mUI/mL), n = 1060 Mean (95% CI)	Nonprotective Antibody Titers (Anti-HAV <10 mUI/mL), n = 28 Mean (95% CI)	*P*
Age (yr)	8.74 (8.72–8.78)	8.62 (8.40–8.84)	NS
Age at vaccination (months)	12.8 (12.7–12.8)	12.7 (12.4–12.9)	NS
Time post vaccination (yr)	7.7 (7.67–7.73)	7.6 (7.37–7.79)	NS

NS indicates nonsignificant.

DISCUSSION

Since 2005 and up to 2013, when this study began, more than 6 million doses of HAV vaccine were administered in the country and national vaccine coverage was above 92% during the whole period. Universal vaccination at 1 year of age against HAV has led in Argentina to an impressive decline in the burden of disease, as evidenced by the decline in reported cases and incidence rates, as well as the absence of HAV-associated liver transplants since March 2007.[21]

In the current study, protective antibody levels against HAV were found in more than 97% of Argentinean children up to 9 years after single-dose vaccination. Our results extend Vizzotti et al's[22] previous findings, and those of Espul et al,[27] that reported a seropositivity rate higher than 93% after 1 dose of vaccine in Argentinean children 4 and 5 years after vaccination, respectively.

As in the authors' previous report,[22] the prevalence of protective antibodies in the current study was similar between regions and, even though when the age of the population ranged from 7 to 10 years, the prevalence of protective antibodies was similar between younger and older children in this analysis.

Some differences were found between current outcomes compared with the previous report, though. The prevalence of protective antibodies was previously associated with kindergarten attendance, and lack of protective antibodies had been associated with terciary/university mother's educational level.[22] To reassess those findings, same variables were included in this present questionnaire but, though demographic and socioeconomic population characteristics resulted similar to that of previous study, neither mother's educational level, assistance to school, number of people per room, excretal disposal type, nor access to tap water was found to be associated, in this current report, with the presence of protective antibodies in the multivariate analysis.

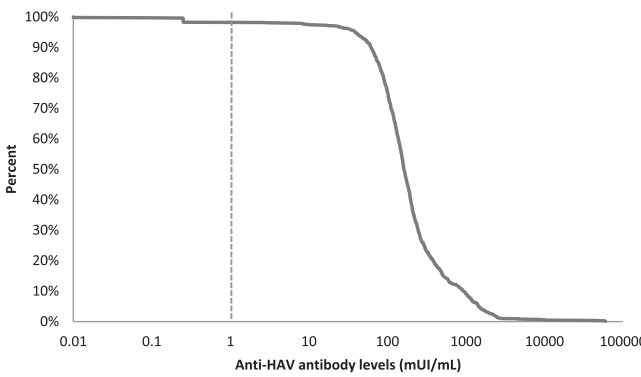

FIGURE 1. Reverse cumulative distribution of anti-HAV antibody levels (mIU/mL).

Anti-HAV GMC of 170.5 mUI/mL found in this study, up to 9 years after vaccination, was rather higher than in our previous report, where anti-HAV GMC was 97.96 mIU/mL (95% CI: 89.21–107.57 mIU/mL), and also higher than in Espul et al's study,[27] which found that anti-HAV GMC was 122.5 mIU/mL (111.2–135.0 mIU/mL), in both cases after a shorter time since vaccination than in the author's current report.[22] Whether differences found in the GMC values are significant, or whether they could be related to technical variations or to a natural boosting effect cannot be answered in this study, as samples were not tested in parallel and we lack information regarding antibody levels immediately after the single-dose priming vaccination to compare. In a seroprevalence study conducted by Mayorga et al[28] in an HAV high endemic area as Nicaragua, authors found that GMC decreased with increasing age, and thereby suggest that such exposures have no boosting effect on antibody levels once protective immunity has been established by natural infection. In contrast, in another longitudinal seroprevalence study conducted by Espul et al[27] in 1- and 2-dose HAV–vaccinated children from Argentina, though GMC decreased along the follow-up period in both groups, there were some individuals who documented a raise in their antibody levels which did not result in a rise in the GMC. Actually, in the group of subjects who received 1 dose of hepatitis A vaccine without booster, anti-HAV titers increased in 34.9%, 13.7%, 29.4% and 21.8% of children at second, third, fourth and fifth year, respectively, and authors suggest a natural boosting effect.[27] To support this possibility, Blanco et al[29] and Yanez et al[30] have documented HAV circulation from environmental surveillance conducted in rivers and sewage samples from different regions of the country in the post universal vaccination era. Regarding this issue, a limitation of our study is that the children's cohort is not the same as our previous one, so this last premise cannot be proved for this trial.

Moreover, there were 4 children with antibody levels >30,000 mIU/mL. These cases possibly represent breakthrough infections but cannot be confirmed as anti-HAV IgM antibodies were not measured and none of them reported to have had clinical disease or contact with any suspected case of hepatitis A infection.

Another limitation is that we could not correlate the rate of seroprotection with the type of vaccine received as different hepatitis A vaccine trade marks were used but these data were not recorded for this study.

After starting with universal single-dose vaccination, there was some concern regarding whether HAV infection and disease could be shifted to older age groups, as it was shown in other countries after significant socioeconomic improvements were made, or after hepatitis A vaccination was introduced with a 2-dose schedule among children.[8,31] However, it is well known that after the introduction of universal vaccination in young children, the older age groups are protected by herd effect. This was the experience, for instance, of Israel when analyzing 13 years of toddlers-only universal routine 2-dose vaccination program.[32] Accordingly, in 10 years of children-only single-dose vaccination program in Argentina, there has been an increase in the proportion of cases in the >14-year-old age group compared with the prevaccination period; however, it still represents a limited number of cases, and a decrease in the absolute incidence rates in all age groups compared with the period before mass vaccination, highlighting the impact of the herd protection.[21]

In conclusion, the universal single-dose HAV vaccination of 1-year-old children in Argentina continues to prove being effective and seroprotection persists up to 9 years after vaccination. Argentina has been a pioneer in the implementation of this strategy, and other countries in the region have also followed the SAGE 2012 recommendation and adopted a similar policy. We believe

that local and periodic seroprevalence studies are vital to give support to this recommendation, and constant passive and active surveillance is crucial to monitor the effectiveness of this innovative strategy.

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