

Single-Dose Hepatitis A Immunization: 7.5-Year Observational Pilot Study in Nicaraguan Children to Assess Protective Effectiveness and Humoral Immune Memory Response

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Background. Universal 2-dose hepatitis A virus (HAV) vaccination of toddlers effectively controls hepatitis A. High vaccine costs, however, impede implementation in endemic countries. To test single-dose vaccination as a possible alternative, we initiated an observational, longitudinal study in Nicaragua, to assess protective effectiveness and—through challenge vaccination—humoral immune memory response.

Methods. After a 2003 serosurvey, 130 originally seronegative children received one dose of virosomal HAV vaccine in 2005, followed by yearly serological and clinical assessments until 2012. After 7.5 years, a vaccine booster was administered. Concurrent antibody screening of patients presenting with hepatitis symptoms documented persistent HAV circulation in the communities studied.

Results. Between serosurvey and vaccination, 25 children contracted hepatitis A subclinically (>8000 mIU/mL anti-HAV). In the remaining 105 children, immunization resulted in anti-HAV levels of 17–572 mIU/mL. Based on the $\geq 15\%$ annual infection risk, an estimated 60% of children were exposed to HAV encounters during follow-up. No child presented with hepatitis symptoms. Serological breakthrough infection (7106 mIU/mL) was documented in 1 child, representing an estimated protective effectiveness of 98.3% (95% confidence interval, 87.9–99.8). Boosting elicited an average 29.7-fold increase of anti-HAV levels.

Conclusions. In children living in hyperendemic settings, a single dose of virosomal HAV vaccine is sufficient to activate immune memory and may provide long-term protection.

Keywords. hepatitis A; single-dose vaccination; hepatitis A vaccine; children; protective effectiveness; long-term follow-up; booster interval; immune memory.

In many developing countries, hepatitis A represents an increasing health issue. An estimated 212 million cases of hepatitis A virus (HAV) infection [1], and 33 million cases of symptomatic illness [2] occurred worldwide in 2005, with some 35 000 estimated deaths, a substantial increase from the 177 million infections estimated for 1990 [1]. In highly endemic, resource-poor countries, hepatitis A causes little symptomatic illness because infections occur mainly in young children, in whom the infection typically remains asymptomatic [1, 3]. However, improvement in hygiene and access to clean

water, as seen in newly industrializing countries, shift the first HAV contact to older age groups. Because older individuals are prone to more severe disease, this leads to a rise in disease burden [1, 2, 4].

This epidemiological transition to lower endemicity and higher disease burden led some endemic countries to implement universal mass vaccination (UMV) of toddlers with 2 doses of inactivated HAV vaccine, the first being Israel in 1999. Israel effectively eliminated hepatitis A within a few years, by targeting young children, the main source of infection, therewith providing herd immunity to older age groups [5]. The United States [6], China [7], and some other industrialized countries [8] decided to protect at first only specific risk groups (toddlers, older children, and teenagers) in certain regions. After a few years of successful regional vaccination campaigns, the United States [6] and China [7] extended their strategies to UMV of toddlers in the mid-2000s, as did Panama and Greece [9, 10]. High vaccine costs, however, impeded for many years a larger-scale implementation of a 2-dose HAV vaccine regimen

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in most endemic countries in need [1, 11, 12]. In 2005, as the first country worldwide, Argentina implemented a single-dose UMV strategy, trusting that protection from 1 dose would last for at least 5–10 years, enough time to eliminate HAV circulation [13].

In the early 2000s, it was shown that a first HAV vaccine dose can efficiently be boosted after 5–8 years in adult travelers [14, 15]. This observation prompted the question of whether a single HAV vaccine dose would suffice to provide lasting protection in individuals living in endemic regions. Building on a cross-sectional, age-stratified hepatitis A serosurvey in 2003/2004 among children and adults in León, Nicaragua [16], we initiated a prospective, observational pilot study in HAV-seronegative children in 2005 to assess the effectiveness and the persistence of immune memory after a single dose of a virosomal HAV vaccine [17].

METHODS

Study Conduct

Our study was based on a serosurvey carried out in León, Nicaragua, in 2003/2004, estimating the annual HAV infection risk, as described elsewhere [16]. HAV-seronegative children identified in the serosurvey were invited to participate in this single-dose HAV vaccine long-term follow-up study. The ethics committee of the National Autonomous University León approved the study. Written informed consent was obtained by the parents of participating children. A standard-of-care hepatitis A vaccine booster dose was to be offered at the end of the study.

A total of 130 children were vaccinated in January 2005 with a single 0.5-mL dose of the virosomal hepatitis A vaccine Epaxal (Crucell Switzerland [formerly Berna Biotech]) [17], followed by serological and clinical assessments after 3 months and then yearly from 2006–2010 and in 2012, to document serological changes and/or clinical signs suggestive of HAV infection. At each yearly visit, parents were asked to report on jaundice or any relevant illness in the previous 12 months. Mid-2012, after an observational period of 7.5 years, a booster dose of an alum-adsorbed hepatitis A vaccine (Havrix Junior or Havrix, depending on age; GlaxoSmithKline) was administered as a standard-of-care procedure. Blood samples were collected before and 4–8 weeks after the booster dose.

To document the continuing HAV circulation in the study area, a viral hepatitis diagnosis project was set up in parallel by the Medical Faculty of the University of León. During 2006–2010, all patients presenting at the community health centers in León with jaundice or other hepatitis symptoms were offered free biochemical tests (liver enzyme and bilirubin measurements) and a serological hepatitis A screening (enzyme-linked immunosorbent assay stripe test), later confirmed by a standard anti-HAV immunoglobulin (Ig) M test [18].

Antibody Testing

Serum samples were stored at -20°C and later shipped to the Institute of Virology, Technical University of Munich, to be tested quantitatively for total anti-HAV antibodies using the microparticle enzyme immunoassay HAVAB 2.0 Quantitative for the AxSYM system (Abbott Diagnostics Division). The lower limit of detection for anti-HAV antibodies was 10 mIU/mL, corresponding to the lowest accepted cutoff for the correlate of protection [6]. High-reacting serum samples (>1000 mIU/mL) were tested for anti-HAV IgM (HAVAB 2.0-M, AxSYM; Abbott). Owing to logistic constraints, the quantitative anti-HAV serology data include the results of 4 testing sessions: 2003 (serosurvey), 2005–2006 (vaccine response and identification of HAV infections), 2006–2010 (follow-up, all serum samples tested in parallel with the same test kit lot), and 2012 (pre- and postbooster assessment of immune memory). The same expert (G. F.) performed all anti-HAV measurements.

Statistical Analysis

Frequencies and percentages of categorical variables were compared using χ^2 or Fisher exact tests as appropriate. Means, standard deviations, medians, and interquartile ranges were reported, and 2-group comparisons were carried out using Wilcoxon-Mann-Whitney tests. One-way analysis of variance was used to compare log-transformed antibody concentrations. Geometric mean concentrations (GMCs) of serum antibodies and their 95% confidence intervals (CIs) were calculated and are given based on log-transformed anti-HAV antibody concentrations.

The age calculation was based on the date of the priming vaccination in 2005. Socioeconomic data from the 2003 serosurvey were used to assess the HAV infection risk between 2003 and 2005. The primary cutoffs of the serological follow-up were set at <1000 mIU/mL for postimmunization (“noninfected”) and ≥ 1000 mIU/mL for infection-related (“infected”) anti-HAV antibody concentrations.

A mixed linear regression model allowing for random intercept and slope was used to ascertain the longitudinal development of the titers incorporating the effect of possibly associated factors, such as age, sex, and socioeconomic status. Vaccine effectiveness was calculated based on the number of breakthrough infections among the vaccinated children and the estimated number of HAV infections among a hypothetical equally sized group of unvaccinated children [19]. All analyses were carried out using Stata software (version 13.1; StataCorp).

RESULTS

Prevaccination Study Phase

A total of 130 children who were seronegative in 2003 received a priming dose of virosomal hepatitis A vaccine (Epaxal) in 2005. For logistic reasons, no second check for seronegativity was performed before this vaccination. The first postvaccination serology 3 months later revealed that in the 13–16 months between

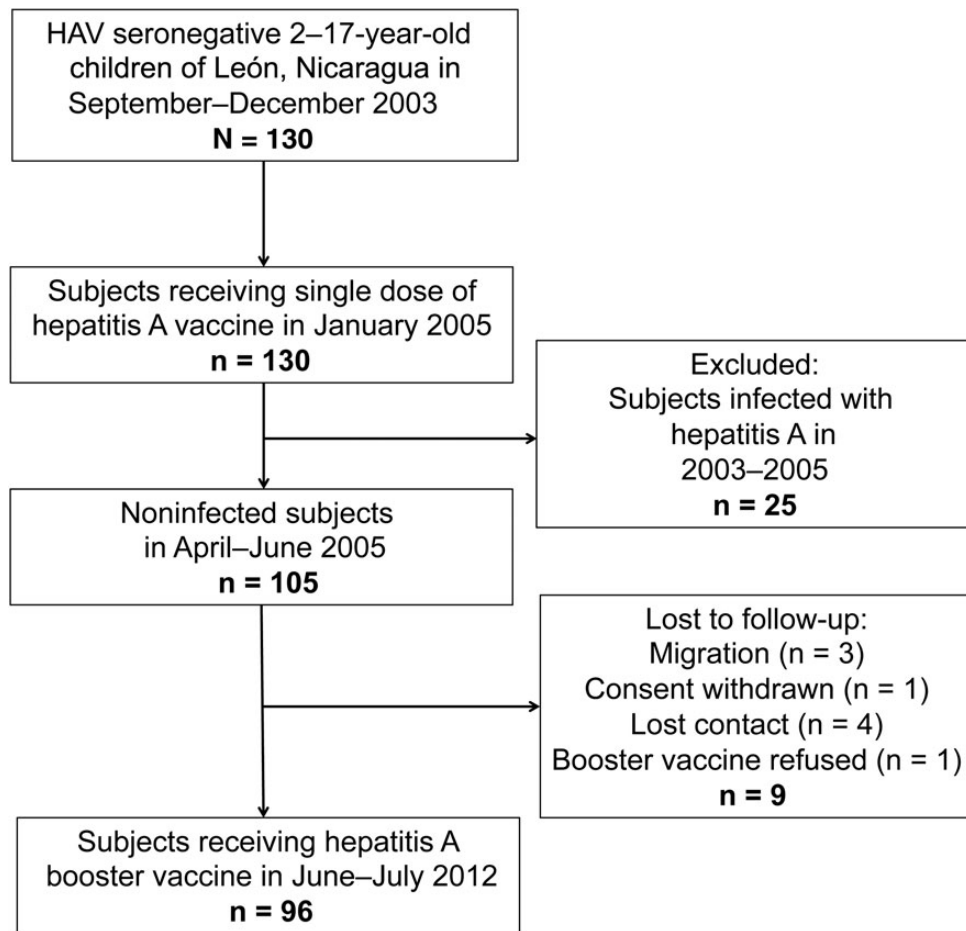


Figure 1. Study flow chart. Excluded subjects were excluded from protective effectiveness and immune memory assessment. Abbreviation: HAV, hepatitis A virus.

the serosurvey at the end of 2003 and the January 2005 single-dose vaccination, 25 (19.2%) of initially seronegative children had contracted hepatitis A subclinically. For the clinical and serological follow-up, the 130 children were, therefore, divided into 2 groups, termed “noninfected” ($n = 105$) and “infected” ($n = 25$, Figure 1). All the infected children had 3-month post-vaccination anti-HAV levels >8000 mIU/mL, with negative anti-HAV IgM in 23 and borderline anti-HAV IgM results in 2 children, whereas in the 105 noninfected children immunization resulted in anti-HAV levels of 17–572 mIU/mL.

The group 3–<6-year-old children had a slightly (not significantly) higher incidence of HAV infection before vaccination compared with those aged <3 or ≥ 6 years. Although reports of no refrigerator, no tap water, or no flush toilet in the household were associated with a slightly higher risk of HAV infection, none of these socioeconomic factors had a significant influence (Table 1).

Demography and Socioeconomic Factors

The median age of the 130 children was 3.6 years (range, 1.7–17 years), and 45.4% were girls (Table 1). The socioeconomic

parameters had not changed significantly between the original data collection in 2003 and the second collection in 2005 (data not shown). In the course of the 7.5-year follow-up, 9 (8.6%) of the noninfected and 4 (16%) of the infected children were lost to follow-up (Figure 1). The 96 noninfected children with complete follow-up did not differ in their demographic or socioeconomic characteristics from the initial group of 105 noninfected children (data not shown).

Serological Follow-up

Immunization of the 105 noninfected children with a single-dose vaccine resulted 3 months later in an anti-HAV antibody concentration (GMC) of 72 mIU/mL (Table 2; Figure 2). Concentrations in these children reached a maximum of 101 mIU/mL in 2007, declining slowly toward 80 mIU/mL in 2012, followed by a 29.7-fold rise (95% CI, 24.5–36.0) to 2399 mIU/mL on challenge. The anamnestic response on boosting was also observed in children whose antibody levels had dropped intermittently or remained <10 mIU/mL for years (Table 3).

Girls responded to the priming vaccination with a higher anti-HAV GMC than boys, a difference that remained borderline

Table 1. Demographic Characteristics (2005) and Socioeconomic Factors (2003)

Characteristic	Subjects, No. (%) ^a			P Value ^b
	All Subjects (N = 130)	Subjects Infected Before 2005 (n = 25)	Subjects Not Infected Before 1st Vaccination (n = 105)	
Sex				
Male	71 (54.6)	14 (56.0)	57 (54.3)	.88
Female	59 (45.4)	11 (44.0)	48 (45.7)	
Age, median (IQR), y	3.63 (2.70–5.30)	3.97 (3.27–5.04)	3.57 (2.64–5.36)	
Age group				
<3 y	45 (34.6)	5 (20.0)	40 (38.1)	.20
3 to <6 y	64 (49.2)	16 (64.0)	48 (45.7)	
≥6 y	21 (16.2)	4 (16.0)	17 (16.2)	
Crowding				
≤2.5 persons/room	57 (43.9)	11 (44.0)	46 (43.8)	.99
>2.5 persons/room	73 (56.1)	14 (56.0)	59 (56.2)	
Refrigerator				
Yes	24 (18.5)	2 (8.0)	22 (20.9)	.16
No	106 (81.5)	23 (92.0)	83 (79.1)	
Water source				
Tap water	109 (83.9)	18 (72.0)	91 (86.7)	.07
Well	21 (16.1)	7 (28.0)	14 (13.3)	
Toilet situation				
Flush toilet	48 (36.9)	6 (24.0)	42 (40.0)	.14
Own latrine	82 (63.1)	19 (76.0)	63 (60.0)	

Abbreviation: IQR, interquartile range.

^a Data represent No. (%) of subjects, unless otherwise noted.

^b Fisher exact, χ^2 test, and Wilcoxon rank sum test comparing infected and noninfected children.

significant throughout the follow-up ($P = .02$ for 2005, $P = .046$ –.10 for 2006–2010, and $P = .03$ before the booster; Figure 3). Whereas the 2 older age groups had fairly similar GMC antibody values throughout the follow-up, the youngest children (aged <3 years), except in 2005, had significantly higher anti-HAV antibody concentrations from 2006 until 2012 (all $P \leq .002$; Figure 3).

Although boys had lower anti-HAV antibody levels after vaccination, they lost anti-HAV antibodies at a slower rate than

girls, even after taking age and socioeconomic status were taken into account in a mixed linear regression model (8.8 mIU/mL less decrease per year; 95% CI, .7–16.9 mIU/mL). Similarly, antibody concentrations rose higher in younger children after vaccination, but with increasing age these concentrations dropped slower (for each year of higher age, 3.1 mIU/mL less antibody decline per year; 95% CI, 1.6–4.6 mIU/mL).

Girls had higher prebooster GMCs than boys (107.5 vs 71.0 mIU/mL). GMC fold-increases after the booster dose were

Table 2. GMCs of Anti-HAV Antibodies, 2003–2012

Visit Year	Infected Before 1st Vaccination		Not Infected Before 1st Vaccination	
	Subjects, No.	Anti-HAV GMC (95% CI), mIU/mL	Subjects, No.	Anti-HAV GMC (95% CI), mIU/mL
2003	25	2.65 (2.12–3.32)	105	2.71 (2.42–3.04)
2005	25	73 724 (49 286–110 281)	104 ^a	72 (63–82)
2006	25	24 088 (15 469–37 510)	103	50 (40–63)
2007	24	32 110 (20 565–50 136)	102	101 (81–127)
2008	23	29 062 (18 651–45 287)	102	96 (77–119)
2009	24	22 580 (15 239–33 457)	101	86 (69–105)
2010	22	19 054 (12 665–28 666)	101	79 (64–98)
2012				
BB	21	18 779 (12 412–28 413)	97	81 (64–101)
AB	0	ND	96	2399 (1044–2815)

Abbreviations: AB, after booster; BB, before booster; CI, confidence interval; GMC, geometric mean concentration; HAV, hepatitis A virus; ND, not determined.

^a No serum available for 1 child whose 2006–2012 sera were always <150 mIU/mL.

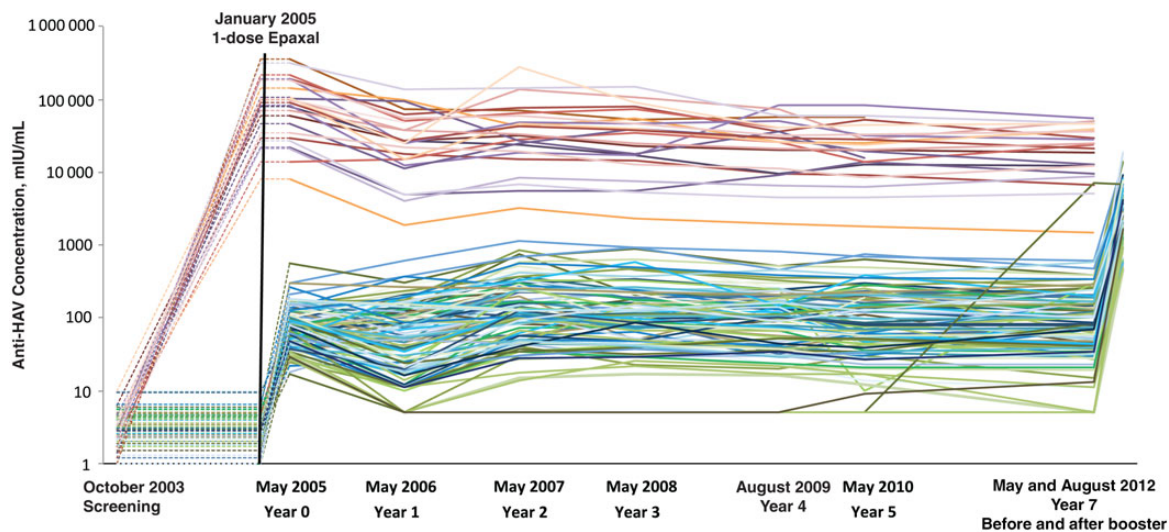


Figure 2. Anti-hepatitis A virus (HAV) antibody concentrations in 2003–2012 in 105 vaccinated and noninfected (green and blue lines) and 25 infected (orange and violet lines) children.

similar in both sexes (27.1 and 24.8; $P = .96$). Thus, girls and boys had postbooster GMCs of 3052 and 1853 mIU/mL, respectively ($P = .002$). Children in the youngest age group had higher prebooster GMCs than those aged 3–<6 or ≥ 6 years (142.5 vs 60.0 and 65.0 mIU/mL, respectively). Postbooster GMCs, however, were similar in all age groups (2301.5, 2490.0, and 2807.5 mIU/mL, respectively; $P = .63$).

Low Responders and Breakthrough Infection

At 3–15 months after the HAV vaccine priming dose, 16 children had no measurable (<10 mIU/mL) or very low (10 to <20 mIU/mL) antibody concentrations. Altogether, 8 children lost detectable anti-HAV antibodies in the course of the follow-up, either intermittently ($n = 4$) or permanently ($n = 4$; Table 3). Before the 2012 booster challenge, 5 children had antibody levels of only 11–19 mIU/mL (data not shown), and 4 had no detectable antibodies at all (Table 3). An asymptomatic

breakthrough infection occurred between 2010 and 2012 in a low responder, an at the time of vaccination (2005) 5.6-year-old girl (anti-HAV 7106 mIU/mL before booster; anti-HAV IgM negative; Table 3).

Clinical Follow-up

No adverse events after vaccination were reported. None of the children ever presented with hepatitis symptoms during the entire follow-up period, including the child with the serological breakthrough infection.

HAV Exposure and Vaccine Effectiveness

The constant circulation of HAV in the community was documented by the hepatitis A screening study run in parallel; throughout 2006–2010, an average of 5–7 cases of acute hepatitis A were diagnosed monthly, mainly in pediatric patients (86% aged 2–10 years) [18]. Figure 4 depicts places of residence

Table 3. Noninfected, Low-Responding Children With ≥ 1 Antibody Concentration <10 mIU/mL During Follow-up

Subject ID	Sex	Age, y	Anti-HAV Antibody Concentration, mIU/mL								
			2005	2006	2007	2008	2009	2010	2012 (BB)	2012 (AB)	
074	F	3.0	28	<10	<10	<10	<10	<10	<10	<10	561
201	M	6.0	32	<10	15	17	16	12	12	<10	450
224	M	5.8	66	<10	30	41	52	51	38	38	1825
236	F	5.9	27	<10	14	26	22	17	<10	<10	1509
237	F	5.6	17	<10	<10	<10	<10	<10	<10	7106	6784
255	M	6.8	35	<10	<10	<10	<10	<10	<10	<10	927
263	M	7.3	18	<10	<10	<10	<10	<10	<10	... ^a	... ^a
295	M	12.9	29	<10	<10	<10	<10	<10	ND	13	1638

Abbreviations: AB, after booster; BB, before booster; HAV, hepatitis A virus; ID, identifier; ND, not determined.

^a Lost to follow-up.

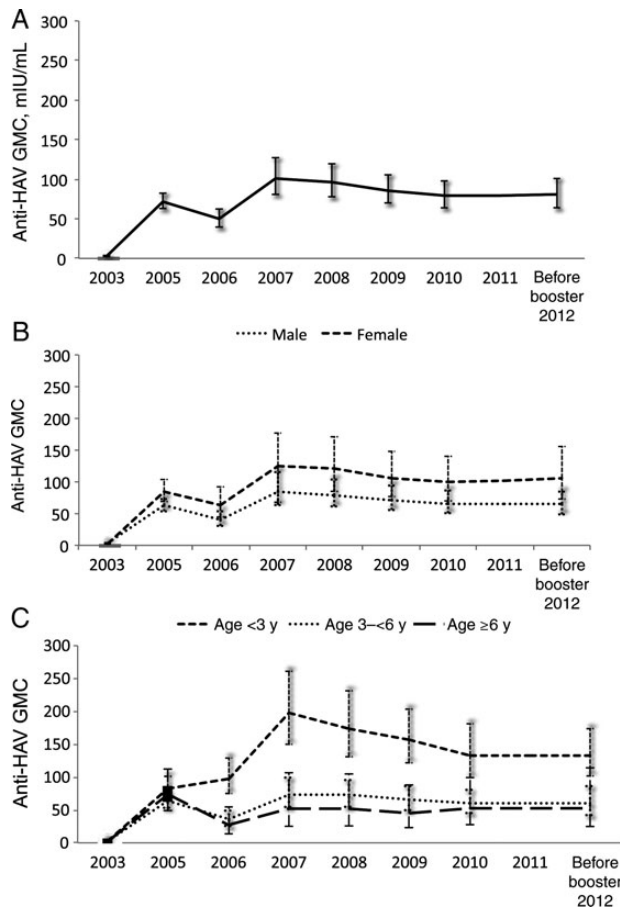


Figure 3. Anti-hepatitis A virus (HAV) geometric mean concentration (GMCs) 2003–2012 (prebooster) among all 105 noninfected children (A), as well as by sex (B) and by age (C); bars represent 95% confidence intervals.

for the acute “community control” hepatitis A case patients as well as for the study participants.

During the 7.5-year postvaccination follow-up, 1 of the 105 vaccinated, HAV-naïve children—a vaccine low responder (subject 237; Table 3)—was subclinically infected with HAV, as indicated by a steep rise in antibody concentration (from <10 up to 7106 mIU/mL), representing an attack rate of approximately 1.0% (0.95% for 1 of 105 children and 1.0% for 1 of 96 with complete follow-up). Based on the known annual risk of infection of (at least) 15% [16, 20], we would have expected 58 wild HAV infections in an unvaccinated group of 96 matched children in 7.5 years, with a probability of 99%. Based on the 1 breakthrough infection in the vaccinated group of 96 children, the vaccine effectiveness with respect to sterilizing immunity worked out to be 98.3% (95% CI, 87.8%–99.8%).

DISCUSSION

The present study and the concurrently conducted community hepatitis A screening [18] confirm a persistently high HAV endemicity in Nicaragua [16, 20]. The 25 infections among 130

children, however, were too few to document a significant influence of any demographic or socioeconomic parameters on the infection risk. The well-mixed geographic distribution of cases over the area of the city of León further emphasizes the high and evenly spread level of HAV circulation the study population was exposed to.

For both infected and noninfected children, the anti-HAV antibody levels declined slightly during the 7.5 years of follow-up. The early loss of measurable antibody within a few years after a single priming dose in 8 noninfected children (8.3%) has been described for up to a third of adult travelers [14, 15, 21] and is somewhat higher than the results found in a large Argentinian study [22], in which antibody levels became unmeasurable within 5 years after a single priming dose in only 2.5% of children [22]. The somewhat variable course of the antibody levels in the vaccinated, noninfected children—falling slightly from 2005 to 2006 and then rising again to a maximum in 2007, before finally declining toward the trough level of 2012—may be ascribed to the fact that not all serum samples could be measured in parallel (see Methods), amplified by the assay variability inherent in immune assay testing [23].

The better immune responses in girls compared with boys and in younger children are known features. Stronger immune responses in favor of the female sex are documented for many different vaccines [24], including inactivated hepatitis A vaccines [25, 26]. The higher antibody response in the youngest age group can be attributed to the lower body volume and therefore a relatively higher vaccine dose. This effect has also been observed in earlier pediatric trials studying 2 dose levels of Epaxal [27].

The calculated 98.3% protection, based on the 1 breakthrough infection among the 96 children followed up and a 15% annual risk of infection, is in line with the known excellent protective efficacy of the virosomal [20] and other inactivated hepatitis A vaccines [1]. To our knowledge, no asymptomatic cases of proved HAV infection after administration of an inactivated HAV vaccine have been reported to date. Only clinical breakthrough infections have been reported, all of them in adult travelers after the priming dose [28–34]. Our subclinical but serologically documented breakthrough infection in an 11-year-old girl can be explained either by the asymptomatic course of hepatitis A in about 50% of children at this age [3] or by a mitigating effect of the priming dose [20, 35] and is quite different from the questionable serological “natural boosters” reported in the literature (see below).

To our knowledge, 2 publications have reported rises in anti-HAV antibody levels observed during serological long-term follow-up of vaccinated children, labeled “natural boosters,” through circulating HAV encounters [22, 36]. In 1 study, antibody levels in 1 of 93 children barely doubled in the second year, compared with the previous value [36]. In the second study, such natural boosters were reported with yearly varying

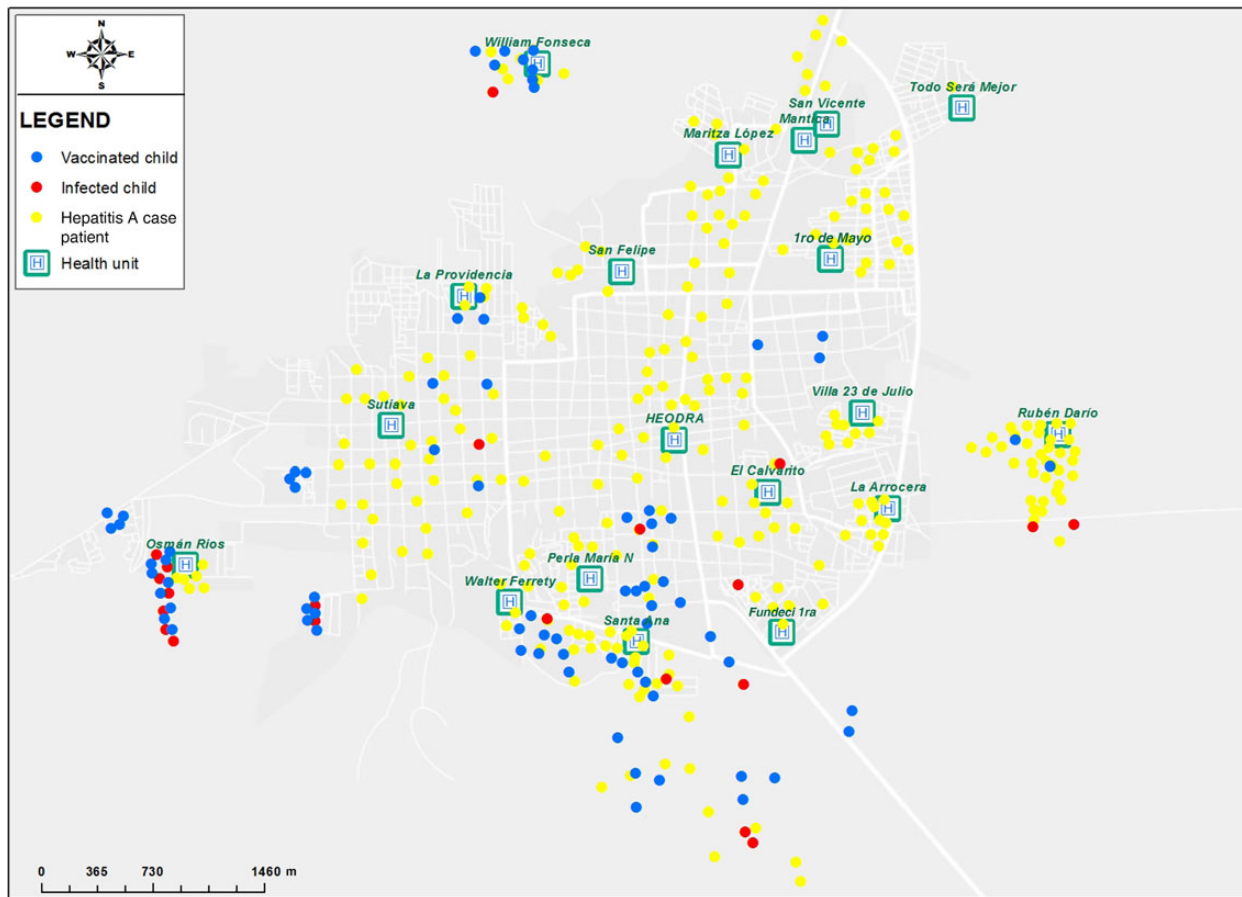


Figure 4. Map of the community of León with the places of residence for the community hepatitis A virus cases observed from 2006 to 2010 [18] and the study participants. The numbers of icons for the infected (*red circles*) and the noninfected (*blue circles*) study participants are somewhat lower than the reported numbers of 25 and 105 children, respectively, because various households had >1 case in the same category. Abbreviation: HEODRA, Hospital Escuela Oscar Danilo Rosales Argüello.

rates in up to one-third of children during 5-year follow-up [22]. The antibody levels rose in the subjects concerned by only 80%–100%, and the consecutive serum samples were not tested in parallel (C. Espul, personal communication). In our experience, testing of consecutive serum samples that is not done in parallel, that is, does not use for all samples the same enzyme immunoassay test kit lot in the same test run, can easily result in up to 50%–100% variations in anti-HAV antibody levels (unpublished data). Contrary to the postulated role of natural boosting in maintaining long-term immunity for certain vaccine preventable infections [37], in our opinion there is no serological natural-booster phenomenon for hepatitis A once anti-HAV immunity has been established, neither after natural infection, nor after successful vaccination. Otherwise in populations living in endemic settings with continuous HAV exposure, constantly high anti-HAV antibody levels would be observed once population-wide seroprotection has been reached. On the contrary, a constant fall in antibody levels from age 15–20 years onward has been documented in an age-stratified, cross-sectional serosurvey in Nicaragua [16].

All children, even those with very low or finally undetectable antibody levels, had a strong humoral immune response after the booster challenge dose, thus confirming the persistence of immune memory, as already documented in adult travelers for up to 11 years after the priming dose [21]. An intact immune memory, despite the loss of any measurable anti-HAV antibodies, has likewise been documented in adults after 1 dose [21], as well as after 2 doses [38]. This strong antibody memory recall response not only reflects residual B-cell response capacity but indicates also that the first vaccine dose elicits an efficient priming of the immune system via an early proliferative T-cell response, as has recently been reported; a single HAV vaccine dose promotes HAV-specific cellular memory immune responses similar to natural infection, and the HAV-specific T-cell immunity induced by primary vaccination persists independently of the circulating antibody levels achieved [39].

Our study has several limitations. No controls to document HAV infections in the population were included; however, HAV infections were monitored at the community level by a hepatitis A screening study conducted in parallel, which

documented persistent HAV circulation in the study area [18]. Seronegativity was not retested before vaccination, leaving space to scrutinize whether all 25 HAV infections between 2003 and 2005 had occurred before vaccination; the anti-HAV IgM testing performed 3 months after vaccination showed, however, that the HAV infections detected serologically had most likely occurred before the first vaccination, because the infection-induced, short-lived (3–6 months) anti-HAV IgM antibodies were either not measurable ($n = 23$) or borderline ($n = 2$) at this time point [4]. Although not all sequential serum samples obtained from each child could be tested in parallel, all anti-HAV measurements were done by the same expert (G. F.), using the same immune assay test system in the same laboratory, thus minimizing the variability inherent to anti-HAV antibody measurement with enzyme immune assays [23].

Originally, the vaccination schedule for inactivated hepatitis A vaccines—that is, a priming dose followed 6–18 months later by a booster dose—was based on early projections of waning antibody levels [1]. From 1999 onward, some countries in transition from higher to lower endemicity started to successfully introduce 2-dose UMV against hepatitis A [5–7]. Because there was evidence that a single hepatitis A vaccine dose can control outbreaks of hepatitis A and induce immune memory [1, 14, 15], as the first country, Argentina successfully introduced the single-dose UMV in 2005 [13, 40, 41].

This single-dose strategy, encouraged by the World Health Organization (WHO) since 2012 [1], seems to be an effective and more affordable option to facilitate the introduction of UMV against hepatitis A [1, 11, 12]. The WHO recommends that HAV vaccination be integrated into the national immunization programs for children aged ≥ 1 year, if indicated on the basis of the country's hepatitis A burden, and that the inclusion of single-dose immunization schedules may be considered, as long as a HAV surveillance and monitoring programs are implemented [1]. According to current WHO data (information taken from the country profiles, last updated 8 January 2016) [42], there are today 4 countries (Argentina, Brazil, Colombia, and Paraguay) using single-dose and 11 countries (Bahrain, Greece, Israel, Mongolia, Panama, Qatar, South Korea, Saudi Arabia, Turkey, United States, and Uruguay) using 2-dose UMV with inactivated HAV vaccines [42]. China has implemented UMV mainly using a single-dose licensed, live attenuated hepatitis A vaccine [7, 42]. Another 11 countries (Australia, Chile, Iceland, Italy, Kazakhstan, Mexico, New Zealand, Moldavia, Russia, Slovenia, and Spain) report vaccinating only certain risk groups in the entire country or only in certain regions [42].

In summary, this prospective, cohort pilot study demonstrated that, in children living in hyperendemic settings, 1 dose of virosomal hepatitis A vaccine is sufficient to activate a solid immune memory and may provide long-term protection, thus supporting the WHO single-dose UMV strategy.

Notes

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Potential conflicts of interest. O. M., C. Hatz, and M. E. in the past have received research funding from Berna Biotech/Crucell for vaccine studies. G. F., in the past and at the time of the study, has provided laboratory support for Berna Biotech/Crucell's hepatitis A vaccine projects. P. V. D. acted as chief and principal investigator for Berna Biotech/Crucell vaccine trials conducted on behalf of the University of Antwerp, for which the University obtains research grants from vaccine manufacturers. C. Herzog was until 2011 an employee of Berna Biotech/Crucell. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. World Health Organization. WHO position paper on hepatitis A vaccines—June 2012. *Wkly Epidemiol Rec* **2012**; 87:261–76.
2. Rein DB, Stevens G, Flaxman A, et al. The global burden of hepatitis A virus in 1990 and 2005. *J Hepatol* **2014**; 60(suppl 1):S303.
3. Armstrong GL, Bell BP. Hepatitis A virus infections in the United States—model-based estimates and implications for childhood immunization. *Pediatrics* **2002**; 109:839–45.
4. World Health Organization. The immunological basis for immunization series: module 18—hepatitis A. Geneva, Switzerland: World Health Organization, **2010**. http://whqlibdoc.who.int/publications/2011/9789241501422_eng.pdf. Accessed 31 March 2016.
5. Levine H, Kopel E, Anis E, Givon-Lavi N, Dagan R. The impact of a national routine immunisation programme initiated in 1999 on hepatitis A incidence in Israel, 1993 to 2012. *Eurosurveillance* **2015**; 20:pii:21040.
6. Centers for Disease Control and Prevention. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *Morb Mortal Wkly Rep* **2006**; 55(No. RR-7):1–23.
7. Fangcheng Z, Xuanyi W, Mingding C, et al. Era of vaccination heralds a decline in incidence of hepatitis A in high-risk groups in China. *Hepat Mon* **2012**; 12:100–5.
8. Hendrickx G, Van Herck K, Vorsters A, et al. Has the time come to control hepatitis A globally? Matching prevention to the changing epidemiology. *J Viral Hepat* **2008**; 15(suppl 2):1–15.
9. Estripeaut D, Contreras R, Tinajeros O, et al. Impact of hepatitis A vaccination with a two-dose schedule in Panama—results of epidemiological surveillance and time trend analysis. *Vaccine* **2015**; 33:3200–7.
10. Mellou K, Sideroglou T, Papaevangelou V, et al. Considerations on the current universal vaccination policy against hepatitis A in Greece after recent outbreaks. *PLoS One* **2015**; 10:e0116939.
11. Ott JJ, Wiersma ST. Single-dose administration of inactivated hepatitis A vaccination in the context of hepatitis A vaccine recommendations. *Int J Infect Dis* **2013**; 17:e939–44.
12. Suwantika AA, Beutels P, Postma MJ. Cost-effectiveness of hepatitis A vaccination in Indonesia. *Hum Vaccin Immunother* **2015**; 10:2342–9.
13. Vizzotti C, González J, Gentile A, et al. Impact of the single-dose immunization strategy against hepatitis A in Argentina. *Pediatr Infect Dis J* **2014**; 33:84–8.
14. Beck BR, Hatz C, Brönnimann R, Herzog C. Successful booster antibody response up to 54 months after single primary vaccination with virosome-formulated, aluminum-free hepatitis A vaccine. *Clin Infect Dis* **2003**; 37:e126–8.
15. Iwarson S, Lindh M, Widerström L. Excellent booster response 4 to 8 years after a single primary dose of an inactivated hepatitis A vaccine. *J Travel Med* **2004**; 11:120–1.
16. Mayorga Perez O, Brinkhof MWG, Egger M, Frösner G, Herzog C, Zwahlen M. Decreasing risk of hepatitis A infection in León, Nicaragua: evidence from cross-sectional and longitudinal seroepidemiology studies. *PLoS One* **2014**; 9:e87643.
17. Bovier PA. Epaxal: a virosomal vaccine to prevent hepatitis A infection. *Expert Rev Vaccines* **2008**; 7:1141–50.

18. Jaisli S, Mayorga O, Frösner G, Herzog C, Zwahlen M. Clinical, serological and epidemiological features of endemic hepatitis A in León, Nicaragua: 315 cases reported May 2006 to June 2010. Presented at: 33rd Annual Meeting of the European Society of Pediatric Infectious Diseases, Leipzig, Germany, 12–16 May, 2015. Poster 101.
19. Halloran ME, Longini IM, Struchiner CJ. Design and interpretation of vaccine field studies. *Epidemiol Rev* **1999**; 21:73–88.
20. Mayorga OP, Herzog C, Zellmeyer M, Loáisiga A, Frösner G, Egger M. Efficacy of virosome hepatitis A vaccine in young children in Nicaragua: randomized placebo-controlled trial. *J Infect Dis* **2003**; 188:671–7.
21. Hatz C, van der Ploeg R, Beck BR, Frösner G, Hunt M, Herzog C. Successful memory response following booster virosome-formulated hepatitis A vaccine delayed up to 11 years. *Clin Vaccine Immunol* **2011**; 18:885–7.
22. Espul C, Benedetti L, Linares M, Cuello H, Rasuli A. Five-year follow-up of immune response after one or two doses of inactivated hepatitis A vaccine given at 1 year of age in the Mendoza Province of Argentina. *J Viral Hepat* **2015**; 22:453–8.
23. Kafatos G, Andrews N, McConway KJ, et al. Estimating seroprevalence of vaccine-preventable infections—is it worth standardizing the serological outcomes to adjust for different assays and laboratories? *Epidemiol Infect* **2015**; 143:2269–78.
24. Cook IF. Sexual dimorphism of humoral immunity with human vaccines. *Vaccine* **2008**; 26:3551–5.
25. Frösner G, Steffen R, Herzog C. Virosomal hepatitis A vaccine: comparing intradermal and subcutaneous with intramuscular administration. *J Travel Med* **2009**; 16:413–9.
26. Pancharoen C, Mekmullica J, Thisyakorn U, Kasempimolporn S, Wilde H, Herzog C. Reduced-dose intradermal vaccination against hepatitis A using an aluminium-free vaccine is immunogenic and can lower costs. *Clin Infect Dis* **2005**; 41:1537–40.
27. Abarca K, Ibáñez I, de la Fuente P, et al. Immunogenicity and tolerability of a paediatric presentation of a virosomal hepatitis A vaccine in Chilean children aged 1–16 years. *Vaccine* **2011**; 29:8855–62.
28. Kurup A, San LM, Yew WS. Acute hepatitis A in a traveler who had received pre-exposure inactivated hepatitis A virus vaccine. *Clin Infect Dis* **1999**; 28:1224–5.
29. Elliot JH, Kunze M, Torresi J. Hepatitis A vaccine failure. *Lancet* **2002**; 359:1948–9.
30. Parment PA, Emilsson H. Hepatitis A after a single dose of an inactivated hepatitis A vaccine. *Scand J Infect Dis* **2002**; 34:634.
31. Junge U, Melching J, Dziuba S. Acute hepatitis A despite regular vaccination against hepatitis A and B. *Dtsch Med Wochenschr* **2002**; 127:1581–3.
32. Taliani G, Sbaragli S, Bartolini A, Tozzi A, Paradisi F, Santini MG. Hepatitis A vaccine failure—how to treat the threat. *Vaccine* **2003**; 21:4505–6.
33. Senn N, Genton B. Acute hepatitis A in a young returning traveler from Kenya despite immunization before departure. *J Travel Med* **2009**; 16:72–3.
34. Mor Z, Lurie Y, Katchman E. A case of hepatitis A vaccination failure in an HIV-positive man who had sex with men in Israel. *Int J STD AIDS* **2012**; 23:529–30.
35. Innis BL, Snitbhan R, Kuanasol P, et al. Protection against hepatitis A by an inactivated vaccine. *J Am Med Assoc* **1994**; 271:1328–34.
36. Chan CY, Lee SD, Yu MI, Wang YJ, Chang FY, Lo KJ. Long-term follow-up of hepatitis A vaccination in children. *Vaccine* **1999**; 17:369–72.
37. Pichichero ME. Booster vaccinations: can immunologic memory outpace pathogenesis? *Pediatrics* **2009**; 124:1633–41.
38. Theeten H, Van Herck K, Van Der Meeren O, Crasta P, Van Damme P, Hens N. Long-term antibody persistence after vaccination with a 2-dose Havrix (inactivated hepatitis A vaccine): 20 years of observed data, and long-term model-based predictions. *Vaccine* **2015**; 33:5723–7.
39. Melgaço JG, Nóbrega Morgado L, Almeida Santiago M, et al. A single dose of inactivated hepatitis A vaccine promotes HAV-specific memory cellular response similar to that induced by a natural infection. *Vaccine* **2015**; 33:3813–20.
40. Yanez LA, Lucero NS, Barril PA, et al. Evidence of Hepatitis A virus circulation in central Argentina—seroprevalence and environmental surveillance. *J Clin Virol* **2014**; 59:38–43.
41. Vizzotti C, Pippo T, Urueña A, et al. Economic analysis of the single-dose immunization strategy against hepatitis A in Argentina. *Vaccine* **2015**; 33(suppl):A227–32.
42. World Health Organization. WHO vaccine-preventable diseases: monitoring system. 2015 global summary. http://apps.who.int/immunization_monitoring/globalsummary. Accessed 31 March 2016.