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Single dose hepatitis A immunisation: 7·5 year observational pilot study in Nicaraguan children to assess protective effectiveness and humoral immune memory response

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Abstract

Background: Universal two-dose hepatitis A virus (HAV) vaccination of toddlers effectively controls hepatitis A. High vaccine costs impede, however, 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: Following a 2003 serosurvey, 130 originally seronegative children received 2005 one dose virosomal HAV vaccine, 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 (>8,000 mIU/mL anti-HAV). In the remaining 105 children, immunisation resulted in 17-572 mIU/mL anti-HAV. Under the ≥15% annual infection risk, an estimated 60% of children were exposed to HAV encounters during follow-up. No child presented with hepatitis symptomatology. Serological breakthrough infection (7106 mIU/mL) was documented in one child, representing an estimated 98.3% (95%CI, 87.9-99.8) protective effectiveness. Boosting elicited an average 29.7-fold increase of anti-HAV levels.

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

INTRODUCTION

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, which is a substantial increase from the 177 million infections estimated for 1990 [1]. In highly endemic, resource poor countries, hepatitis A causes little symptomatic illness as 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 industrialising countries, shift the first HAV contact to older age groups. As 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 two 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 USA [6], China [7] and some other industrialised countries [8] decided to protect at first only specific risk groups (toddlers, older children, teenagers) in certain regions. After a few years of successful regional vaccination campaigns, the USA [6] and China [7] extended in the mid 2000s their strategies to UMV of toddlers, as did Panama and Greece [9, 10]. High vaccine costs impeded, however, for many years a larger scale implementation of a two-dose HAV vaccine regimen 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 one dose would last for at least five to ten 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 five to eight years in adult travellers [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 following one 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 offered 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.

130 children were vaccinated in January 2005 with one 0·5 mL dose of the virosomal hepatitis A vaccine Epaxal® (Crucell Switzerland AG, formerly Berna Biotech) [17], followed by serological and clinical assessments after 3 months, and then yearly from 2006 to 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, according to age Havrix Junior® or Havrix® (GlaxoSmithKline, Belgium) was administered as a standard of care procedure. Blood samples were collected prior to and four to eight 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 to 2010 all patients presenting at the community health centres in León with jaundice or other hepatitis symptoms were offered free biochemical (liver enzymes, bilirubin) tests and a serological hepatitis A screening (ELISA stripe test, Orgenics, Israel), later confirmed by a standard anti-HAV IgM test [18].

Antibody testing

Sera were stored at -20ºC 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 from Abbott Diagnostics Division, Wiesbaden, Germany. The lower limit of detection was 10 mIU/mL anti-HAV, corresponding to the lowest accepted cut-off for the correlate of protection [6]. High reacting sera (>1000 mIU/mL) were tested for anti-HAV IgM (HAVAB 2·0-M, AxSYM, Abbott). Due to logistic constraints the quantitative anti-HAV serology data are composed of the results of four different testing sessions: 2003 (serosurvey), 2005 to 2006 (vaccine response and identification of HAV infections), 2006 to 2010 (follow-up, all sera tested in parallel with the same test kit lot), and 2012 (pre/post booster assessment of immune memory). The same expert (GF) performed all anti-HAV measurements.

Statistical analysis

Frequencies and percentages of categorical variables were compared using Chi-squared or Fisher's exact tests as appropriate. Means, standard deviations, medians and interquartile ranges were reported and two-group comparisons were carried out using Wilcoxon-Mann-Whitney tests. One-way analysis of variance (ANOVA) was used to compare log-transformed antibody concentrations. Geometric mean concentrations (GMCs) of serum antibodies and their 95% confidence intervals 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 cut-offs of the serological follow-up were set at <1000 mIU/mL for post-immunisation ('not infected') and at ≥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 titres 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 13·1 (Stata Corp. LP, Texas, USA).

Role of the funding source

The industry funder had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript. The corresponding author had full access to the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Pre-vaccination study phase

130 children who were seronegative in 2003 received a priming dose of virosomal hepatitis A vaccine (Epaxal®) in 2005. Due to logistic reasons no second check of the seronegativity was carried out prior to this vaccination. The first postvaccination serology three months later revealed that in the 13 to 16 months between 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 two groups, termed 'not infected' (n=105) and 'infected' (n=25, Figure 1). All the 'infected' children had three months postvaccination anti-HAV levels of >8,000 mIU/mL, with negative anti-HAV IgM in 23 and borderline anti-HAV IgM results in two children, while in the 105 'not infected' children immunisation resulted in anti-HAV levels of 17 to 572 mIU/mL.

The group of the three to six year old children had a slightly higher (not significant) incidence of HAV infection prior to vaccination as compared to children aged less than three and more than six years. Although 'no refrigerator', 'no tap water' and 'no flush toilet' in the household was associated with a slightly higher risk of HAV infection, none of these socioeconomic factors had a significant influence (Table 1).

Demography & 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 collection in 2003 and the second data collection in 2005 (data

not shown). In the course of the 7·5 year follow-up, nine (8.6%) of the not infected children and four (16%) infected children were lost to follow-up (Figure 1). The 96 not infected children with complete follow-up did not differ in their demographic or socioeconomic characteristics from the initial group of 105 not infected children (data not shown).

Serological follow-up

Immunisation of the 105 not infected children resulted three months after the single dose vaccination in an anti-HAV antibody concentration (GMC) of 72 mIU/mL (Table 2, Figure 2). These children reached a maximum of 101 mIU/mL in 2007, declining slowly towards 80 mIU/mL in 2012, followed by a 29·7-fold rise (95%CI, 24·5-36·0) to 2399 mIU/mL upon challenging. The anamnestic response was also observed in children in whom the antibodies had dropped intermittently or remained below 10 mIU/mL for years (Table 3).

Girls responded to the priming vaccination with a higher anti-HAV GMC than boys, a difference which remained borderline significant throughout the follow-up (p=0·022 for 2005, p=0·046–0·101 for 2006-2010 and p=0·033 pre-booster, Figure 3). Whereas the two older age groups had fairly similar GMC antibody values throughout the follow-up, the youngest children (below three years) showed, except for 2005, significantly higher anti-HAV antibody concentrations from 2006 until 2012 (all p≤0·002, Figure 3).

Although male children developed lower anti-HAV antibodies after vaccination, they lost anti-HAV antibodies at a slower rate than females even after taking age and socioeconomic status into account in a mixed linear regression model (8·8 mIU/mL (95%CI 0·7–16·9) less decrease per year). Similarly, younger children rose to higher antibody concentrations after vaccination, but with increasing age

antibody concentrations dropped slower (for each year of higher age 3·1 mIU/mL (95%CI 1·6–4·6) less antibody decline per year).

Girls had higher pre-booster titres than boys (107·5 *vs*. 71·0 mIU/ml). Both sexes developed similar fold increases to the booster dose (27·1 and 24·8, p=0·96). Thus girls had a post-booster titre of 3052 mIU/ml and boys of 1853 mIU/ml (p=0·0023).

Children in the youngest age group had the highest pre-booster titres compared to children aged three to six and above six years of age (142·5 vs. 60·0 and 65·0 mIU/ml). Post-booster titres were, however, similar in all age groups (2301·5, 2490·0 and 2807·5 mIU/ml, p=0·63).

Low responders and breakthrough infection

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

An asymptomatic breakthrough infection occurred between 2010 and 2012 in a low responder, originally (2005) 5·6 years old girl (anti-HAV 7106 mIU/mL pre-booster, anti-HAV IgM negative, Table 3).

Clinical follow-up

No adverse events following 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 on average five to seven cases of acute hepatitis A were diagnosed monthly in mainly pediatric patients (86% aged 2–10 years) [18]. In Figure 4 the acute 'community control' hepatitis A cases' as well as the study participants' places of residence are depicted.

During the 7·5-year post-vaccination follow-up, one of the 105 vaccinated, HAV naive children – a vaccine low responder (ID 237, Table 3) – was subclinically infected with HAV, as indicated by a steep rise in antibody concentration (<10 up to 7106 mIU/mL), representing an attack rate of approximately 1·0% (0·95% for 1/105 children and 1·0% for 1/96 children with complete follow-up). Calculating with the known (at least) 15% annual risk of infection [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 one 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 were, however, too few to document a significant influence of any of the demographic or socioeconomic parameters on the infection risk. The well-mixed geographical distribution of cases over the area of the city of León further emphasises the high and evenly spread level of HAV circulation the study population was exposed to.

For both, the infected and the not infected 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 eight (8·3%) not infected children has been described for up to a third of adult travellers [14, 15, 21] and is somewhat higher than the results found in a large Argentinian study [22]. In the latter study only 2.5% of children converted to unmeasurable antibody levels within 5 years after a single priming dose [22]. The somewhat variable course of the antibody levels in the vaccinated, not infected children - slightly falling from 2005 to 2006 and then rising again to a maximum in 2007, before finally declining towards the trough level of 2012 - may be ascribed to the fact that not all sera could be measured in parallel (see method section), amplified by the assay variability inherent to immune assay testing [23].

The better immune responses in girls as compared to boys and in younger children are known features. Stronger immune responses in favour 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 paediatric trials studying two different dose levels of Epaxal® [27].

The calculated 98.3% protection, based on the one breakthrough infection among the 96 children followed-up and an 15% annual risk of infection, is in line with the known excellent protective efficacy of the virosomal [20] and of other inactivated hepatitis A vaccines [1]. To our knowledge, no asymptomatic cases of proven HAV infection following administration of an inactivated HAV vaccine have been published to date. Only clinical breakthrough infections have been reported, all of them in adult travellers 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, two publications report on rises in anti-HAV antibody levels observed during serological long-term follow-ups of vaccinated children, labelled 'natural boosters' through circulating HAV encounters [22, 36]. In one publication, antibody levels in one of 93 children barely doubled in the second year compared to the previous value [36]. In the second study such 'natural boosters' were reported with yearly varying rates in up to one third of children during a 5-year follow-up [22]. The antibodies rose in the subjects concerned by 80-100% only and the consecutive sera were not tested in parallel (C.Espul, personal communication). In our experience, not parallel testing of consecutive sera, i.e. not using the same EIA 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 serologic '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 to 20 years onwards has been documented in an age-stratified, cross-sectional serosurvey in Nicaragua [16].

All children, even the ones with very low or finally undetectable antibody levels, had a strong humoral immune response following the booster challenge dose, thus confirming the persistence of immune memory, as already documented in adult travellers 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 one [21], as well as after two 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 it 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 community level by a hepatitis A screening study conducted in parallel which documented persistent HAV circulation in the study area [18]. Seronegativity was not re-tested prior to vaccination, leaving space to scrutinise whether all 25 HAV infections between 2003 and 2005 had occurred prior to vaccination; the anti-HAV

IgM testing performed three months after vaccination showed, however, that the HAV infections detected serologically had most likely occurred prior to the first vaccination, as the infection-induced, short-lived (3-6 months) anti-HAV IgM antibodies were not measurable (n=23) or borderline (n=2) at this time-point [4]. Although not all sequential sera obtained from each child could be tested in parallel, all anti-HAV measurements were done by the same expert (GF), using the same immune assay test system in the same laboratory, thus minimising the variability inherent to anti-HAV antibody measurement with enzymatic immune assays [23].

Originally, the vaccination schedule for inactivated hepatitis A vaccines, i.e. a priming dose, followed six to eighteen months later by a booster dose, was based on early projections of waning antibody levels [1]. From 1999 onwards, some countries in transition from higher to lower endemicity started to successfully introduce two-dose UMV against hepatitis A [5-7]. Since 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 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 programmes for children aged ≥1 year if indicated on the basis of the country's hepatitis A burden, and that the inclusion of single-dose immunisation 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 8.1.2016) [42] there are today four countries (Argentina, Brazil, Colombia, Paraguay) using single-dose and 11 countries (Bahrain, Greece, Israel, Mongolia, Panama, Qatar, South Korea, Saudi

Arabia, Turkey, USA, Uruguay) using two-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, Spain) report to vaccinate only certain risk groups in the entire country or only in certain regions [42].

In summary, this prospective, cohort pilot study demonstrates that in children living in hyperendemic settings one 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:

Congress presentation. Part of the material contained in this manuscript has been presented as a poster (# 514) at the 33rd Annual Meeting of the European Society of Pediatric Infectious Diseases in Leipzig, 2015.

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Potential conflict of interest. OM, ME and CHa received in the past research funding from Berna Biotech/Crucell for vaccine studies; PVD acted as chief and principal investigator for Berna Biotecch/Crucell vaccine trials conducted on behalf of the University of Antwerp, for which the University obtains research grants from

vaccine manufacturers; CHe was until 2011 an employee of Berna Biotech/Crucell; GF was in the past and at the time of the study providing laboratory support for Berna Biotech/Crucell's hepatitis A vaccine projects; UP, SBü, VKJ and SBa have no interests to declare. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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Tables and figures

Figure 1:

Study flow chart. Excluded*: subjects excluded from protective effectiveness and immune memory assessment

Figure 2:

Anti-HAV antibody concentrations 2003-2012 in 105 vaccinated, not-infected (green/blue lines) and 25 infected (orange/violet lines) children. HAV: hepatitis A virus

Figure 3:

Anti-HAV GMCs 2003 – 2012 (pre-booster) of all 105 not infected children (A), as well as by sex (B) and by age (C); the bars indicate the 95% CIs. HAV: hepatitis A virus; GMC: geometric mean concentration; CI: confidence intervals

Figure 4:

Map of the community of León with the places of residence of the community hepatitis A cases observed from 2006 to 2010 [18] and of the study participants. The number of icons for the infected [red filled circles] and the not infected [blue filled circles] study participants is somewhat lower than the reported number of 25 and 105 children, respectively, as various households had more than one case of the same category.

Table 1:

Demographic characteristics (2005) and socioeconomic factors (2003)

	All subjects	**Subjects infected before 2005**	**Subjects not infected before 1st vaccination**	**P values[§]**
	N = 130	N = 25	N= 105	
	n (%)	n (%)	n (%)	
Sex				
Male	71 (54·6)	14 (56·0)	57 (54·3)	0·88
Female	59 (45·4)	11 (44·0)	48 (45·7)	
Age (years)				
Median [IQR][#]	3·63 [2·70-5·30]	3·97 [3·27 - 5·04]	3·57 [2·64- 5·36]	
<3	45 (34·6)	5 (20·0)	40 (38·1)	0·20
3 to <6	64 (49·2)	16 (64·0)	48 (45·7)	
≥6	21 (16·2)	4 (16·0)	17 (16·2)	
Crowding[$]				
≤2·5	57 (43·9)	11 (44·0)	46 (43·8)	0·99
>2·5	73 (56·1)	14 (56·0)	59 (56·2)	
Refrigerator				
Yes	24 (18·5)	2 (8·0)	22 (20·9)	0·16
No	106 (81·5)	23 (92·0)	83 (79·1)	
Water				
Tap water	109 (83·9)	18 (72·0)	91 (86·7)	0·07

Well	21 (16·1)	7 (28·0)	14 (13·3)	
Toilet situation				
Flush toilet	48 (36·9)	6 (24·0)	42 (40·0)	0·14
Own latrine	82 (63·1)	19 (76·0)	63 (60·0)	

[§] Fisher exact, Chi squared test, Wilcoxon Ranksum test comparing infected and non-infected children; [#] IQR: interquartile ranges; [$] Crowding: subjects per room

Table 2:

Geometric mean concentrations (GMC) of anti-HAV antibodies 2003 -2012

	Infected before 1st vaccination			**Not infected before 1st vaccination**		
Visit year	**n**	**GMC[$]**	**95% CI[£]**	**n**	**GMC**	**95% CI**
2003		2·65	2·12-3·32		2·71	2·42-3·04
2005	25	73724	49286-110281	104	72	63-82
2006	25	24088	15469-37510	103	50	40-63
2007	24	32110	20565-50136	102	101	81-127
2008	23	29062	18651-45287	102	96	77-119
2009	24	22580	15239-33457	101	86	6-105
2010	22	19054	12665-28666	101	79	64-98
2012*	21	18779	12412-28413	97	81	64-101
2012#	0	nd	nd	96	2399	1044-2815

[$] GMC: geometric mean concentration; [£] CI: confidence interval; *pre-booster; #post-booster; nd: not done; HAV: hepatitis A virus

Table 3:

Not infected, low responding children with one or more instances of antibody concentrations <10 mIU/mL during follow-up

ID	Sex	Age	Anti-HAV[$] antibody concentrations (mIU/mL)							
		(years)	2005	2006	2007	2008	2009	2010	2012*	2012#
074	f	3·0	28	<10	<10	<10	<10	<10	<10	561
201	m	6·0	32	<10	15	17	16	12	<10	450
224	m	5·8	66	<10	30	41	52	51	38	1825
236	f	5·9	27	<10	14	26	22	17	<10	1509
237	f	5·6	17	<10	<10	<10	<10	<10	7106	6784
255	m	6·8	35	<10	<10	<10	<10	<10	<10	927
263	m	7·3	18	<10	<10	<10	<10	<10	-- §	-- §
295	m	12·9	29	<10	<10	<10	<10	nd	13	1638

[$] HAV: hepatitis A virus; * pre-booster; # post-booster; § lost to follow-up; nd: not done

HAV seronegative, 2-17 years old children of León, Nicaragua in September to December 2003
N = 130

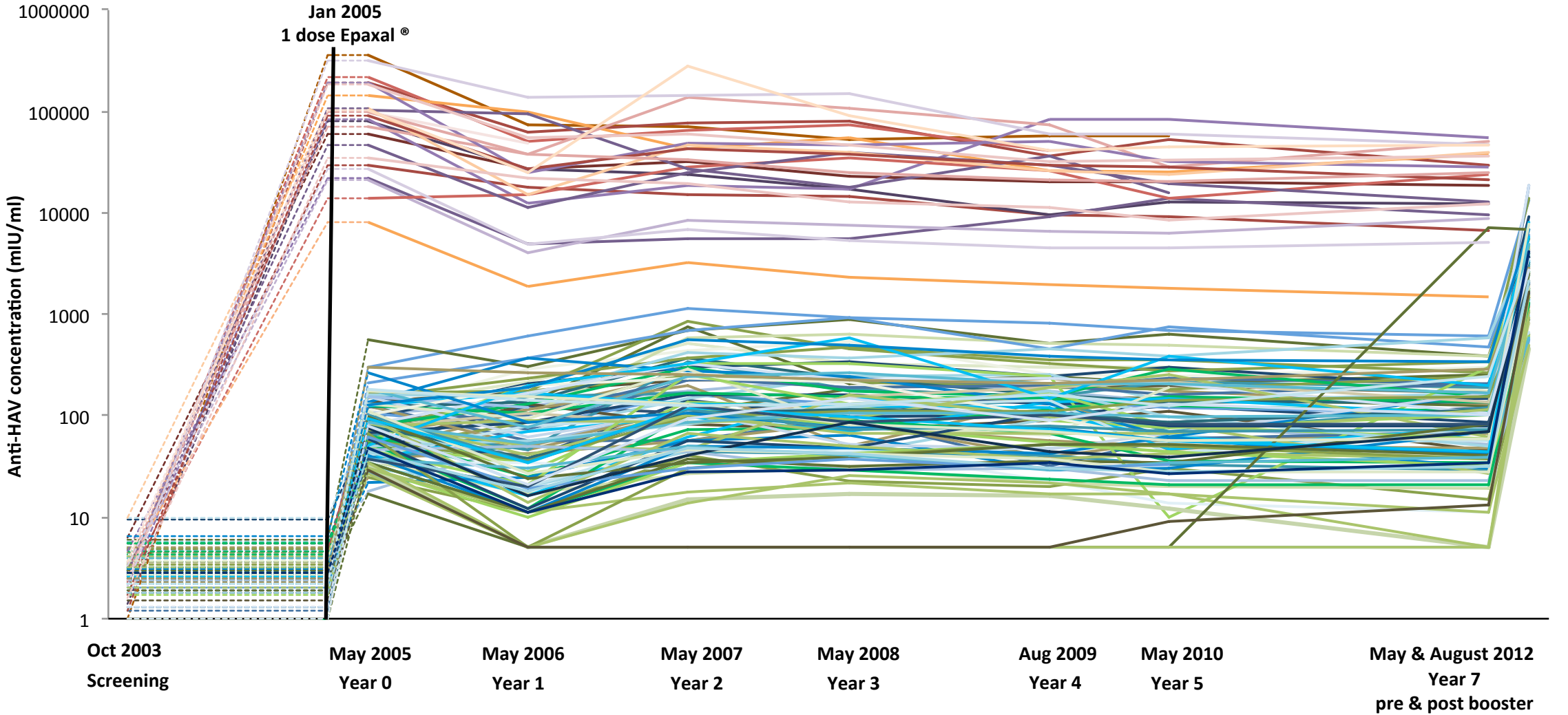
Subjects receiving single dose of hepatitis A vaccine in January 2005
N = 130

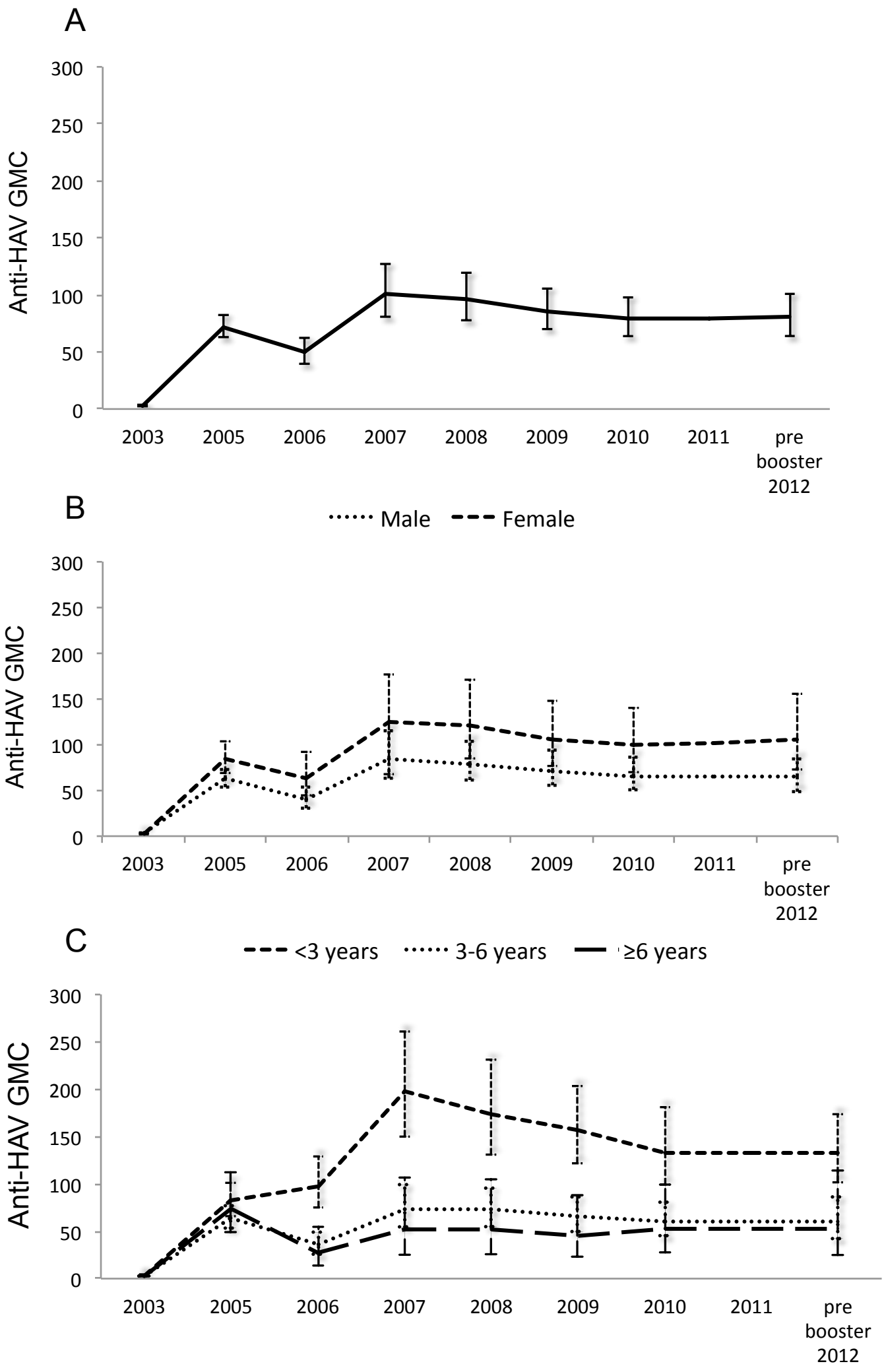
Excluded*:
Subjects infected with hepatitis A between 2003-2005
N = 25

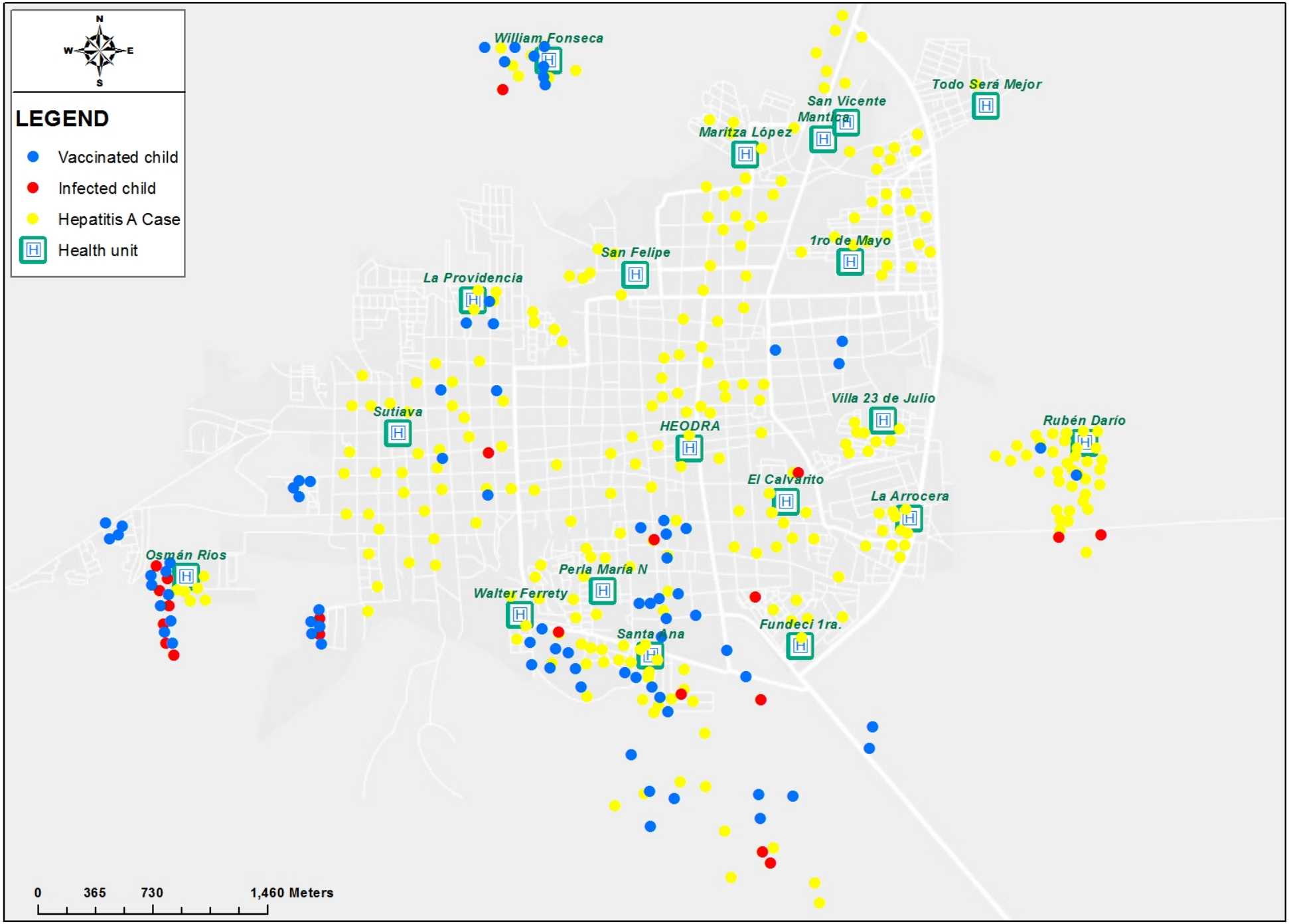
Non-infected subjects in April-June 2005
N=105

Lost to follow-up:
Migration (3)
Consent withdrawn (1)
Lost contact (4)
Booster vaccine refused (1)
N = 9

Subjects receiving hepatitis A booster vaccine in June-July 2012
N = 96


Jan 2005
1 dose Epaxal ®
Anti-HAV concentration (mIU/ml)
1000000
100000
10000
1000
100
10
1
Oct 2003
Screening
May 2005
Year 0
May 2006
Year 1
May 2007
Year 2
May 2008
Year 3
Aug 2009
Year 4
May 2010
Year 5
May & August 2012
Year 7
pre & post booster


A
Anti-HAV GMC
300
250
200
150
100
50
0
2003
2005
2006
2007
2008
2009
2010
2011
pre booster 2012
B
Male
Female
Anti-HAV GMC
C
<3 years
3-6 years
≥6 years
Anti-HAV GMC


N
W
E
S
LEGEND
Vaccinated child
Infected child
Hepatitis A Case
Health unit
William Fonseca
Todo Será Mejor
San Vicente
Maritza López
1ro de Mayo
San Felipe
La Providencia
Sutiava
Villa 23 de Julio
HEODRA
Rubén Darío
El Calvario
La Arrocera
Osmán Rios
Perla María N
Walter Ferrety
Santa Ana
Fundeci 1ra.
0
365
730
1,460 Meters