International Journal of Infectious Diseases (2008) **12**, 183—189

http://intl.elsevierhealth.com/journals/ijid

Eight years of hepatitis B vaccination in Colombia with a recombinant vaccine: factors influencing hepatitis B virus infection and effectiveness

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Received 4 December 2006; received in revised form 5 June 2007; accepted 21 June 2007
Corresponding Editor: William Cameron, Ottawa, Canada

KEYWORDS
Hepatitis B;
Vaccine;
Effectiveness;
Epidemiology;
Colombia

Summary
Objective: To evaluate the effectiveness of a recombinant hepatitis B vaccine used in endemic areas of Colombia, as well as risk factors associated with hepatitis B virus (HBV) infection and carriage after vaccine introduction.
Methods: A cross-sectional study was carried out in urban and rural areas of the Colombian Amazon, a highly endemic area for hepatitis B infection. Children under 12 years of age and their mothers were selected for the study using one-stage cluster sampling ($N = 2145$) and were examined for HBV serological markers and antibodies against surface antigen (anti-HBs).
Results: There has been a reduction of 60—75% in the prevalence of HBV infection and hepatitis B surface antigen (HBsAg) carriage since HBV vaccination was introduced. Receiving the first dose of HBV vaccine at more than two months after birth was one of the factors associated with HBV carrier status. Maternal HBV infection was also associated with infection in the child.
Conclusions: The recombinant Cuban hepatitis B vaccine has contributed to the reduction of the infection in this highly endemic area, though further efforts are required to improve timely vaccination for children at high risk.

Introduction

Hepatitis B virus can lead to acute and chronic infection. Infection is transmitted by blood exposure, by sexual intercourse, during childbirth, and in other ways that are not

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1201-9712/$32.00
doi:10.1016/j.ijid.2007.06.010

completely understood during early childhood. More than 300 million people are chronically infected with hepatitis B virus (HBV) worldwide, with Asia and Africa carrying the highest burden of the disease. Several areas in South America have also been described as zones of high endemicity.[1–3]

There are five areas in Colombia where more than 70% of the population has been infected with HBV. They are situated on the Caribbean Coast, the Uraba Gulf, the Pacific Coast, the Amazon Basin, and the Catatumbo River on the border with Venezuela. A serological study made in 1980 on 60% of the Colombian population found an HBsAg prevalence of from 3% to 8% across different age groups. From these findings it is estimated that there are 600 000 HBV carriers and 4 000 000 HBV infected people in Colombia.[4–9]

Following World Health Organization (WHO) recommendations, in 1992 Colombia introduced a control program for HBV infection using a recombinant vaccine. This program did not include maternal screening or hepatitis B immune globulin (HBIG) for children born to hepatitis B surface antigen (HBsAg) positive mothers. In order to assess the effectiveness of this intervention, we carried out a sero-epidemiological survey in the Colombian Amazon of children less than 10 years of age and their mothers.

Methods

Study design

A one-stage cross-sectional survey was carried out in rural and urban areas of the Colombian Amazon including Leticia, Puerto Nariño, Araracuara, and Puerto Santander. Leticia and Puerto Nariño are located in the area of the Amazon River, and around half of the population (26 200 people) live in rural areas and are very poor. Araracuara and Puerto Santander are small villages (<1500 inhabitants) situated on the banks of the Caquetá River, in the central area of the department, and have been described as very high risk areas for HBV in previous studies.[10] Most of the Amazon's people belong to aboriginal tribes and live in precarious socio-economic and sanitation conditions. The target population was composed of children between 1 and 12 years of age, since they were in the age group eligible for HBV vaccination.

Sample size

It was calculated that 2239 children would be needed to estimate an HBsAg positive prevalence of 2% or more, with an absolute precision of 0.8% and a design effect of 2.0. Children were selected with probability proportional to size.

In urban and rural Leticia, we randomly selected 72 clusters out of 183. In rural Puerto Nariño, 11 out of 18 villages were randomly selected with probability proportional to size. In urban Puerto Nariño, Araracuara, and Puerto Santander, all households were included given their small size.

Logistical aspects of fieldwork

Two health promoters visited every selected household and recorded the family's socio-economic conditions (crowding, running water, social security). If one or more eligible children were found, interviewers asked parents for a vaccination card. If a card was available, interviewers recorded the number of vaccine doses received and the dates of vaccination. The child's parents/guardians were asked to answer a questionnaire on general risk factors for hepatitis B infection, such as family and personal antecedents of clinical hepatitis and family history of fulminant hepatitis, cirrhosis, or hepatocellular carcinoma. The parents' level of education, breastfeeding practices, mother's age at birth of first child, mother's age at birth of the child in question, the child's number of siblings, ethnic group, and the site where the child was born were also recorded. Following signature of an informed consent form by the child's parent/guardian, a blood sample was drawn from the child and the mother. Sera were separated, stored frozen, and shipped to the Virology Laboratory of the National Institute of Health for analysis of hepatitis B virus markers.

Hepatitis B vaccination characteristics

Until 2001, Colombia used a recombinant hepatitis B vaccine (Heberbiovac HB) manufactured in Cuba, which contains 20 μg of HBsAg.[11] Three doses of HBV vaccine were recommended at 2, 4, and 6 months of age. During the study period no screening program was in place to detect HBsAg positive pregnant women and no HBIG was offered.

Definitions for vaccination status

Completely vaccinated children were those holding a vaccination card where three doses of HBV vaccine could be identified. Those who failed to fulfill these criteria were considered as incompletely vaccinated. Children without written evidence of vaccination were analyzed as a separate category.

Serological analysis

The children's sera were processed for hepatitis B surface antigen (HBsAg), total antibody to core antigen (anti-HBc), antibody against core antigen IgM (IgM anti-HBc), and antibodies against surface antigen (anti-HBs). All sera were processed initially for HBsAg and anti-HBc. All sera positive for both HBsAg and anti-HBc were further tested for IgM anti-HBc. Anti-HBs titers were measured in a random sample of children negative for both HBsAg and anti-HBc. Serological screening was done using ELISA, while HBsAg carriage was confirmed by neutralization. A similar scheme was used to process the sera of the mothers.

Data handling and analysis

Epidemiological data were entered into several databases using EpiInfo 6.04. The prevalence of HBV infection and HBsAg positivity was calculated taking into account the complex survey design.

We compared the present prevalence of HBV infection and HBsAg carriage against prevalence found by previous studies carried out in the same areas before the onset of the vaccination program.[10] Only prevalence in rural areas was compared, since they were the only areas included in previous data. Differences were stratified by age, sex, and place of study.

Variables potentially related to HBV infection and HBsAg carriage were divided into the following categories: (1) child-related variables (age, sex, gender, birth order, qualification of the person delivering the child, and ethnic group); (2) vaccination characteristics (time in days between birth and the first dose of hepatitis B vaccine, time between first and second dose, and time between second and third dose); (3) mother-related variables (mother's serological status for hepatitis B, place where mother was born, and mother's history of clinical hepatitis). A separate analysis was done for HBV infection and HBsAg carriage with each of these categories. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using bivariate and multivariate analysis (logistic regression). Variables found to be statistically related to HBV infection or HBsAg carriage were included in a final multivariable model combining variables from all categories. Logistic regression models were built using the svy and the logistic command with cluster and strata options.

Levels of anti-HBs antibody titers were related to several variables (age, gender, ethnic group, breastfeeding, days between doses of vaccine, and days between last dose and sampling date) using two approaches. For the first one, titers were treated as a continuous variable. Geometric means and medians of titers were calculated for every category of independent variable. Mean or median differences were tested with non-parametric techniques such as the Kruskall–Wallis test. Those variables where anti-HBs differed statistically across categories ($p < 0.1$) were included in a multivariable linear regression model.

In the second approach, children were categorized into two groups according to their anti-HBs titers. One included children with titers of <10 IU/l (not protected) and the second, children with titers above that level. Bivariate analysis was done calculating ORs and 95% CIs as a measure of the degree of the association. Those variables found related ($p < 0.1$) in the bivariate analysis were included in a logistic model.

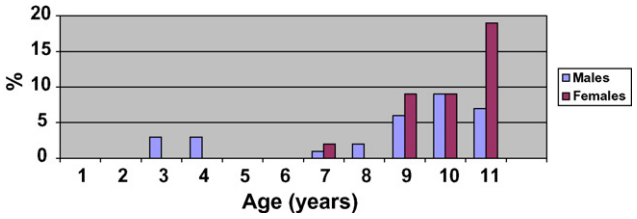

Figure 1 HBsAg prevalence (%) in rural areas by age and sex; all children.

Results

Among 2145 children, the overall prevalence of HBV infection was 6.2% (95% CI 4.7–7.9) and HBsAg carriage was 1.1% (95% CI 0.4–1.8%). The prevalences of infection and carriage were higher in rural than in urban areas (9.2% and 2.6% vs. 2.6% and 0.17%, respectively) and in children 8 years and older, especially girls (see Figure 1).

A reduction (60–75%) in the prevalence of HBV infection and carriage was observed for the period since the introduction of the vaccine, especially in the most endemic areas, namely Araracuara. This reduction remained after stratifying by age, sex, and place (see Table 1).

Ethnic origin, birthplace, and mother's serological status were found to affect the risk of HBV infection. Compared to non-Indians, those belonging to an Indian tribe such as Ticunas had a higher prevalence of HBV infection (OR = 2.4, 95% CI 1.2–4.6). Belonging to an Indian group other than Ticunas or Huitotos was associated with an even higher risk when compared to non-Indians (OR = 4.6, 95% CI 2.4–8.6). Not being born in a hospital or health center increased the risk of infection two-fold (OR = 2.4, 95%

Table 1 Prevalence of hepatitis B infection and HBsAg found before and after the introduction of the hepatitis B vaccine, by age group and place

Variable	% Prevalence before vaccination[a] (*n*)	% Prevalence after vaccination[b] (*n*)	% Reduction (95% CI)
Overall			
Children 5–9 years, infection	32% (334)	9% (493)	72 (59–78)[c]
Children 10–14, infection	66% (189)	25% (160)	62 (49–72)[c]
Male children 5–9 years, infection	34% (157)	9% (247)	73 (59–83)[c]
Female children 5–9 years, infection	30% (177)	10% (246)	67 (48–78)[c]
Male children 10–14 years, infection	85% (144)	19% (87)	78 (64–85)[c]
Female children 10–14 years, infection	76% (135)	32% (72)	58 (40–70)[c]
Children 5–9 years, HBsAg+	7% (334)	2% (495)	71 (35–84)[c]
Male children 5–9 years, HBsAg+	8% (157)	2% (247)	75 (26–90)[c]
Araracuara and Puerto Santander			
Children 5–9 years, infection	39% (111)	9% (125)	77 (54–86)[c]
Children 10–12 years, infection	87% (75)	28% (75)	68 (53–78)[c]
Children 5–9 years, HBsAg+	9% (111)	2% (125)	73 (6–93)[d]

[a] Year 1992 (Ref. 10).
[b] Year 1999 (including only children from rural areas).
[c] $p < 0.001$.
[d] $p < 0.05$.

Table 2 Variables related to HBsAg prevalence, urban and rural areas

Variable	People studied (*n*)	HBsAg prevalence (*n* (%))	OR (95% CI)	Adjusted OR (95% CI)
Child-related variables				
Age groups (years)				
1—3	435	1 (0.2)	1.0	1.0[a]
4—5	435	3 (0.7)	2.6 (0.3—27.2)	2.1 (0.2—22.8)
6—7	456	2 (0.3)	1.1 (0.1—11.5)	0.8 (0.1—10.0)
8—11	580	19 (3.0)	10.0 (1.2—90.8)	6.0 (0.6—65.4)
Birth received by				
Doctor/nurse	1091	2 (0.2)	1.0	1.0[a]
Other	801	23 (2.7)	13.0 (3.0—57.0)	6.5 (1.5—27.6)
Number of siblings				
1—5	1210	8 (0.5)	1.0	1.0[a]
6—20	356	11 (3.2)	6.2 (2.1—18.2)	3.3 (1.1—10.0)
Mother-related variables				
Mother anti-HBc+				
Yes	782	21 (2.4)	6.6 (1.8—25.1)	3.5 (1.0—11.8)[b]
No	895	3 (0.4)	1.0	1.0
Vaccine-related variables				
Time from birth to first dose				
0—60 days	495	1 (0.2)	1.0	1.0[c]
61—183 days	198	2 (1.2)	6.6 (0.5—90.4)	7.2 (0.5—115.1)
184—665 days	194	1 (0.6)	2.2 (0.1—40.4)	2.6 (0.1—50.0)
666—3253 days	199	7 (3.5)	8.9 (0.9—88.2)	12.5 (1.2—125.7)
Unknown	830	14 (1.2)	4.1 (0.5—35.4)	6.6 (0.6—66.4)
Time from first to second dose				
28—35 days	467	3 (0.6)	1.0	1.0[c]
36—62 days	202	4 (1.9)	3.3 (1.2—8.9)	1.6 (0.7—3.8)
63—1877 days	426	7 (1.6)	2.8 (0.9—11.2)	1.2 (0.4—3.6)
Unknown	811	11 (1.0)	1.3 (0.3—4.7)	0.8 (0.2—3.7)

[a] OR adjusted by the remaining variables in the same category.
[b] OR adjusted by age group and place where mother was born.
[c] OR adjusted by age group, 'birth received by', number of siblings, mother's infection status, and area (rural and urban).

CI 1.5—4.1). Being born to an anti-HBc antibody positive woman also increased the risk of infection (OR = 1.7, 95% CI 1.1—2.6). None of the vaccine-related variables were found to be associated with being HBV infected.

The variables associated with being a carrier were: not being born in a hospital or health center (OR = 6.5, 95% CI 1.5—27.6), living with more than five siblings (OR = 3.3, 95% CI 1.1—10.0), and being born to an anti-HBc positive mother (OR = 3.5, 95% CI 1.0—11.8) (Table 2).

There were 1407 (66%) children with data available on hepatitis B vaccination. Ninety-one percent of them (1277) had completed the basic scheme for hepatitis B (three doses). No statistical differences were found in HBsAg prevalence (1.2% vs. 0%) or infection (6% vs. 0%) between children with complete or incomplete vaccination. Interestingly, HBsAg prevalence among children without a vaccination card was very similar to the prevalence in documented vaccinated children (1.1%). Time from birth to first dose of HBV vaccine was related to HBsAg carriage even after controlling for mother- and child-related variables. Receiving the first dose of vaccine two months or later following birth was related to an increase in the risk of HBsAg carriage, especially among those receiving the vaccine after two years of life (OR = 12.5, 95% CI 1.2—125.7) (see Table 2). Figure 2 shows the time differences between birth and first dose for HBsAg carriers and non-carriers. The time between first and second dose was longer in carriers from rural areas. Receiving the second dose 35 days or more after the first was associated with a two-fold increase in the risk of HBsAg carriage (OR = 2.3, 95% CI 1.4—3.8). This association remained after controlling for age, mother's serological status, and number of siblings.

Levels of anti-HBs were quantified in a sample of 263 non-infected (HBsAg negative and anti-HBc negative) vaccinated children. Titers ranged between 0 and 10 000 IU/l, 13% did not have detectable anti-HBs, and 20% had titers under 10 IU/l. Anti-HBs geometric mean and median concentrations were 105 IU/l and 172 IU/l, respectively. Twelve percent of

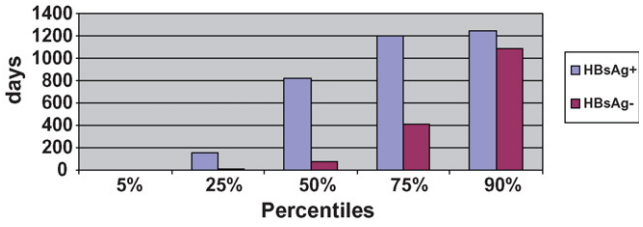

Figure 2 Time (days) from birth to first hepatitis B vaccine dose by HBsAg status; percentiles.

Table 3 Anti-HBs levels by selected variables

Variable	Number without anti-HBs (%)	Number with anti-HBs >10 mIU/ml (%)	Number with anti-HBs ≥1000 mIU/ml (%)[a]	Anti-HBs GMT [Median]
Time between birth and first dose	$p = 0.03$		$p = 0.03$	$p = 0.01$
0–14 days	19 (33)	39 (67)	3 (5)	33 [68]
15–60 days	10 (19)	42 (81)	4 (8)	81 [169]
61–183 days	4 (8)	45 (92)	9 (18)	174 [153]
184–665 days	9 (20)	36 (80)	9 (20)	66 [110]
666–3253 days	10 (17)	49 (83)	14 (24)	145 [357]
Time between first and second dose	$p = 0.24$		$p = 0.14$	$p = 0.09$
13–35 days	24 (23)	80 (77)	11 (11)	64 [119]
36–62 days	8 (17)	38 (83)	4 (9)	85 [148]
63–147 days	10 (17)	50 (83)	11 (18)	93 [142]
148–1877 days	9 (18)	41 (82)	11 (22)	126 [223]

[a] This category is included in the total number of those with anti-HBc levels >10 mIU/ml. GMT, geometric mean titer.

children had anti-HBs levels above 1000 IU/l. Two variables were related to lack of anti-HBs: time interval since receiving third dose and time interval from birth to first dose. Children receiving their first dose within 14 days of birth had lower levels of anti-HBs (geometric mean titer (GMT) = 33 IU/l vs. 66–174 among the other groups) (Table 3).

Discussion

There is a paucity of information in Latin America on the effectiveness of hepatitis B vaccination programs, although Brazil, Peru, Cuba, and Colombia have introduced them.[12,13] Recently, a Peruvian group of researchers made an assessment of the impact of the introduction of hepatitis B vaccine in an endemic area of Peru, where they found a reduction in the prevalence of HBV infection of from 83% to 92%. Compliance with schedules was higher in the Peruvian study, since it was a pre-introductory evaluation where the vaccination process was supervised by researchers.[14,15] The impact of the vaccination program in our study is quite clear. Using the representative population of the study of Cristancho[10] for the pre-vaccination era, there has been a highly significant reduction in both infection and carriage, which is similar to the reduction found in other recent studies in Pacific countries (80%).[16]

Children over 8 years of age were more likely to be found to be HBsAg carriers than those under 8 years. Children of that age are more likely to refuse vaccine injections; also children of that age were more likely to already have been infected when HBV vaccination was started.[10] Higher prevalence in older children has been found in other areas where the hepatitis B vaccine has been introduced into the Expanded Program on Immunization (EPI).[17]

No child born to a carrier mother was found to be an HBsAg carrier in our study (0/24) despite none of them receiving HBIG. This is low compared with other studies. Wilson et al.[16] found that 27% of children born to an HBeAg positive mother were HBsAg positive (13/48). This may be due to differences in the chances of perinatal transmission in different parts of the world, an issue related to the HBV-DNA levels, which also vary among HBsAg positive mothers across regions.[18–21] Mothers in Asian countries are more infectious to their children than mothers in Africa (due to the higher levels of HBV-DNA shown by Asian mothers), and our results suggest that the perinatal transmission risk is lower for Amerindian children compared to the Asians or Africans; this has been found by others in the Brazilian Amazonia. Miranda Braga et al. found an HBeAg prevalence of only 6% among 70 HBsAg positive Indians examined in the Brazilian Amazon, which also suggests lower levels of HBV-DNA in HBsAg carriers for this region.[22] It should also be recognized that ethnic and genetic characteristics can play a role in explaining the differences in perinatal transmission.[17,22,23]

Of note, in our study we also looked into the risk factors for hepatitis B infection other than the mother's serological status. It is clear from our results that some of these factors are still important predictors of HBV infection after vaccine introduction. The number of siblings and the child's birth circumstances were the most important individual variables identified. Children whose birth was not attended by a nurse/doctor were twice as likely to be found HBsAg positive as those who were attended by a doctor or nurse. This relation was even stronger in rural areas where the OR increased to more than 10-fold. It is likely that being born in a hospital or health center results in receiving the first dose of hepatitis B vaccine closer to the birth date. Another potential explanation is that practices around home-attended births increase the child's risk of HBV infection.

We were able to demonstrate that delay in dose delivery is associated with a higher likelihood of being HBsAg positive. This aspect has not been frequently addressed by other studies, either because vaccination timing was standardized (clinical trials) or because there are few studies focusing on time between doses. Former studies focusing on anti-HBs titers as the main outcome have concluded that hepatitis B vaccine can be delivered following almost any schedule (0-1-3, 0-2-4, 0-1-6 months, etc.).[24,25] In contrast, our results show that while longer intervals can produce higher anti-HBs titers, they might favor infection leading to the HBsAg carrier status. Ruff et al. in Indonesia, showed that a delay of more than a week between birth and first dose was associated with a higher risk of becoming HBsAg positive.[26] Other recent studies have found no relation between HBV carriage and vaccine delays, though they have found an important proportion of children receiving vaccine doses later than recommended.[16]

Our study did not find statistical differences in infection rates between completely and incompletely vaccinated children; however, one study in Taiwan found completeness of vaccination as a predictor for HBsAg carriage.[27] This may be due to a lack of power in our sample since few carriers and infected children were found. Also, children known to be born to an HBsAg positive mother might have been pursued more actively to ensure more adequate vaccination, thus leading to a bias in our study. This last explanation is not probable, since no screening for HBsAg in mothers was in place in the Amazon from the start of the vaccination program to the moment when our study was carried out. It could also be related to the role that perinatal transmission has in the Amazon, which is smaller than in Taiwan.

Other studies have found high proportions of vaccinated children without detectable anti-HBs levels in countries where regular programs are carried out. Poovorawan et al. and Wilson et al. found prevalences in children without anti-HBs to be similar to or even higher (21—50%) than those found in our study (26%).[16,28] These figures are higher than those reported by clinical trials, and potential explanations include cold chain factors and type of vaccine. A recombinant vaccine is used in the Amazon, and it has been shown that plasma vaccine is more immunogenic especially when anti-HBs levels are compared shortly after completing the scheme.[29,30]

The health workers poor knowledge of hepatitis B vaccination guidelines might be related to the proportion of children without anti-HBs in the present study. Most health workers said that the vaccine should be applied in the buttock, which is known to lead to a lower serological response.[31,32]

Our study has several limitations. This is a cross-sectional study where exposure and infection is measured at the same time. This might impair causal inference since the temporal relationship between vaccination and infection is not accurate, i.e., several vaccinated children may already have been infected before they received the vaccine. Another limitation is that no measurements of HBV-DNA levels were done in the present study, which may be a residual confounder not controlled in the analysis.

In summary, the process of implementing a new vaccine against hepatitis B in the Colombian Amazon has been successful. HBV vaccine has reached a high coverage especially among children born after the implementation of the program, though adherence to vaccine schemes should be improved. There has been an important reduction in the prevalence of HBV infection and HBsAg carriers, especially among children aged 0 to 5 years, despite HBIG or screening of pregnant women not being available. However, new vaccination strategies should be introduced in order to ensure an adequate and timely access of the population to the vaccine, especially in rural villages. Based on our recommendations, the Amazon Health Service has started a serological screening for pregnant women aimed at providing more adequate HBV vaccination schemes to children born to infected or HBsAg carrier mothers.

Acknowledgements

This study was supported in part by the Pan American Health Organization (PAHO) through the Division of Human Development. It also received economic support from other areas of the same agency such as the Colombian Office and from the Division of Vaccines and Immunizations. The principal author was supported by a grant from Colciencias while studying in London. We also wish to thank the people living in the urban and rural communities of the Amazon in Colombia who agreed to participate in this research, and the field workers of the Amazon Health Secretary who enthusiastically collaborated with this study.

Conflict of interest: No conflict of interest to declare.

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