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Hepatitis B infection control in Colombian Amazon after 15 years of hepatitis B vaccination. Effectiveness of birth dose and current prevalence

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ABSTRACT

Background: Hepatitis B virus (HBV) infection is highly endemic in the Colombian Amazon basin. In Colombia, the universal hepatitis B vaccination in that area has been active since 1993. The program targets children aged under five years. Newborns receive at least three doses, and in 2001, HBV vaccine birth dose was included. This study aimed to evaluate the advances on HBV control in the Colombian Amazon.
Methods: A population-based cross-sectional study was conducted in children less than 11 years old in rural areas of the Colombian Amazon, in order to assess the current levels of HBV prevalence and evaluate the effectiveness of HBV vaccination. Participants were selected from villages scattered along the Amazon, Putumayo and Loretoyaco Rivers. Blood samples were taken from children. All the samples were examined for surface antigen (HBsAg) and IgG antibodies against core antigen (AntiHBc) of HBV. Data on HBV vaccination status and other risk factors were also collected.
Results: Blood samples from 1275 children were included in the study. The positivity for IgG AntiHBC and HBsAg was 3.8% and 0.5%, respectively. It was observed that receiving a dose of HBV vaccine within 48 h after birth decreased the risk of HBV infection and carriage by 95%. Being born to an AntiHBc positive mother increased 8 times the risk of HBV infection (OR = 7.8 CI 95% 3.3–10.2) and 7 times the risk of HBsAg carriage (OR = 6.6 CI 95% 2.1–10.1).
Conclusion: The prevalence of HBV infection and HBsAg carriage continues to decrease among children living in the Colombian Amazon. The high protective effectiveness of an HBV birth does suggest that perinatal transmission is important in endemic areas of Latin America, an aspect that has not been fully studied in the region.

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1. Introduction

Currently, there are around 240 million chronic carriers of HBsAg around the world, which are at the highest risk of developing chronic liver diseases such as primary liver cirrhosis and hepatocelular carcinoma [1]. Most countries in Latin America and the Caribbean (LAC) are classified as low endemic areas, but there are some high endemicity spots as well [2]. One of the most extensively studied high endemic areas in LAC is the Amazon basin, an area that is shared by Brazil, Colombia, Venezuela, Peru, and Ecuador. In the pre-vaccine era, the prevalence of HBsAg among adults was above 7–26%, especially in rural areas, and there were frequent outbreaks of fulminant hepatitis related to hepatitis delta virus (HDV) [3–5].

A highly effective Hepatitis B vaccine is available since the 1980s [6]. Its effectiveness to decrease the prevalence of HBV infection, HBsAg carriage, and primary liver cancer has been

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demonstrated in high endemic areas, in particular in Africa [7] and South-East Asia [8–10].

The World Health Organization (WHO) recommends that all countries should use a scheme that includes a birth dose (administered within the first 24 h after birth) plus at least two doses using a monovalent or combined vaccine following the schedules with diphtheria, pertussis and tetanus vaccine (DTP) [11].

Despite LAC countries started universal hepatitis B vaccination in the mid-1990s, there are still gaps in knowledge about the effectiveness of HBV vaccination in high endemicity areas in the region [12]. Epidemiology of hepatitis B was extensively studied in the pre-vaccine era, but by contrast, there are only three studies published so far describing it after vaccine introduction [4,13,14]. Furthermore, these studies have produced conflicting results. Braga et al. 2012 [13], found a low effectiveness of HBV vaccination, around 30%, while De la Hoz et al., 2008 [14], and Cabezas et al., 2014 [4] reported a substantial reduction of HBsAg carriage and HBV infection in the post-vaccine era.

The Colombian Ministry of Health introduced universal HBV vaccination in the Amazon in 1993-targeting newborns and children <5 years- using a three doses scheme with a monovalent vaccine manufactured in Cuba. In 2001, it changed to a schedule composed of a birth dose, with a monovalent vaccine, and three subsequent doses using a pentavalent vaccine (DTP + *Haemophilus influenza* type b (Hib) + HBV). In 1999, a serological survey study found a reduction of ≈70% in HBV infection and HBsAg carriage among children of urban and rural areas in the Colombian Amazon state despite health services' struggle to provide timely access to HBV vaccine for rural population [14].

Vaccinating rural communities in the Amazon state also was introduced in 1993, and it was considered an area of high endemicity for hepatitis which has the highest endemicity for hepatitis B. In this area, it was a particularly challenging because of geographical, logistical, and cultural constraints. Several studies have shown that most children have received at least two doses of HBV vaccine, but timely coverage is low, especially with the birth dose [15,16]. The present study aimed to assess the current epidemiology of HBV infection and carriage among Amazonian children born after the vaccine policy shift-introduction of birth dose and switch to pentavalent vaccine, in order to inform the public and health authorities on the advances to control HBV infection, and the current challenges faced by the policy. It presents the magnitude of HBV infection as well as the estimated effectiveness of HBV vaccination.

2. Methods

A serological and epidemiological survey was carried out in order to assess the advances in the control of HBV infection in the Colombian Amazon state, Southeast, Colombia. The study comprised two main objectives: (1) the evaluation of the coverage with HBV vaccine including the proportion of children vaccinated with a timely birth dose and, (2) the prevalence of HBV infection and HBsAg carriage in children under 11 years old, and their mothers and the effectiveness of HBV vaccination strategies. Results from the HBV vaccination coverage and factors hampering vaccination timeliness have been already been studied [15,16].

The main population methods used in this study have been described previously [15]. The present study presents the specific methodological aspects related to the serological study. The survey was conducted between July 2011 and March 2012. This study was approved by the Ethical Committee of the School of Medicine at the National University.

2.1. Study site

The study was carried out in three municipalities of the Amazonas state: Leticia, Puerto Nariño, and Tarapaca. The municipalities were selected based on previous knowledge of HBV infection epidemiology in this region. Leticia and Puerto Nariño concentrated the largest proportion of the region's population, while Tarapaca was included because it represents the isolated rural areas of the Amazon where a substantial proportion of HBV infection occurred in the pre-vaccine era [5].

2.2. Sample size

The sample size was calculated to assess an HBsAg carriage prevalence of 1% within a margin of error of 0.5% and 95% of confidence level. A sample size of 1300 children would be needed to fulfill those assumptions. At the end, 1275 children were included in the serological study.

2.3. Study population and field procedures

Rural areas from the municipalities selected for the study were included in the sampling due to previous data showing that rural areas bear the largest burden of HBV infection [5,14]. In Leticia, 12 out of 21 rural communities were included in the study. In Puerto Nariño, 17 out of 20 were included, and 8 out of 13 rural communities were included in Tarapacá.

The community approval was obtained from community leaders after explaining the objectives and procedures of the study. A field work team, composed of bacteriologists and nurses visited every household with children of an eligible age in every selected village once approval was obtained from the community. At every household with eligible children, parents were asked to give the permission for their children to participate in the study. If they agreed to participate, then they were invited to sign a consent form. They were specifically asked to allow for a blood sample to be drawn from children and children's mothers, as well to answer a questionnaire on the vaccination status of the children, the potential risk factors for HBV infection. Children less than 11 years of age with HBV vaccination status data information were considered eligible for the study.

2.4. Serological outcomes

A person with HBV infection was defined as someone who has a positive result for the serological Anti-HBc marker by ELISA. Anti-HBc-positive and HBsAg-negative markers were considered as a resolved infection with or without lifelong immunity. An HBsAg carrier was defined as someone with a positive result for the serological marker HBsAg after 6 months of age. The positivity for HBsAg was considered as a marker for asymptomatic carrier status, which in the presence of other markers may show active or chronic active infection. Individuals with a positive result to both, anti-HBc and HBsAg were considered as a frequent infection and a risk of transmitting the disease (Table 1).

2.5. Exposures

The main exposures were: (1) HBV vaccination status with a separate analysis for timely vaccination with the birth dose of HBV vaccine. When vaccination records were not available or difficult to read, the Expanded Program on Immunization (EPI) databases was consulted (SIVIGILA, Instituto Nacional de Salud Colombia). These databases are updated by the EPI nurses and contain vaccination data from every child from rural areas.

Table 1
Hepatitis B markers used in the study.

Infection phase	Serological markers			
	HBsAg	Anti HBc	DNA	ORFs
Late incubation period	+	−	−	−
Acute hepatitis first phase	+	+	+	+
Acute hepatitis second phase	+	+	−	−
Acute hepatitis third phase or recovery with immunity	−	+	−	−
Chronic hepatitis	+	−/+	+	+
Healthy carrier	+	+	−/+	+

A timely birth dose should be given in the first 24 h after delivery as recommended by the WHO [11]; however, vaccination cards showed dates of birth delivery and HBV birth dose, but they lacked the data on the exact hour of both variables. Therefore, in the present study, a timely birth dose was defined a one administered on the same date of delivery or the day after, which means delivered in the first 48 h after birth. A timely HBV vaccination scheme was defined as a timely birth dose plus 3 doses of pentavalent vaccine at 2, 4 and 6 months with at least 30 days interval between each dose.

2.6. Laboratory procedures

A blood sample was taken from both children. The serum was obtained and stored at −70 °C. All samples were evaluated for serological markers anti-HBc and HBsAg by Enzyme-Linked ImmunoSorbent Assay (ELISA). Samples anti-HBc+ were analyzed by PCR for HBV genome detection and sequencing. DNA extraction was performed from 200 μL of serum sample using a commercial kit (QIAmp DNA mini kit, QIAGEN, Germany); some modifications of centrifugation times and additional washings were included. A serum HBV+ with a viral load of 50.000 IU/mL and a sample of liver tissue from a bank of explants tissues HBV+ were used as positive controls in the PCR. Three PCRs were carried out for amplification of the three regions of HBV ORF S (S, PreS1, and PreS2) [17]. The S region was amplified using a nested PCR with 5 U of Biolasa polymerase (Bioline, UK), 2.5 mM of each dNTPs, 10× reaction buffer, 25 mM of $MgCl_2$, 10 μM of YS1 and YS2 primers and 5 μL of DNA in a final volume of 50 μL for the first round. For the second round, 2 μl of first round PCR product was added to 23 μl of the reaction mixture under the same conditions as the first round using primers S3s/S3as. Amplification of region PreS1 was carried out with P1/P2 primers for the first round and 2440p/58n primers for the second round, in a final volume of 50 μL 164 and 25 μL, respectively. PreS2 region was amplified using P3006f/P213r primers in a final volume of 25 μL for both rounds. The PCRs products were visualized in 2% agarose gels stained with SYBR green at a 10 μg/mL concentration. The gels were photo-documented with the 2UV Transilluminator Digital Imaging System equipment (UVP, USA).

2.7. Statistical analysis

An Excel database was constructed to store the variables collected on the field. All the statistical analysis was done using STATA 12. The frequency of serological outcomes and main exposures were described using proportions and 95% confidence intervals (95% CI) proportions. For other covariates, proportions, means or medians with 95% confidence intervals (95% CI) were used according to the probability distribution. Participants in the study were selected by village, so theoretically their characteristics tended to be clustered at that level. Therefore, the survey svy commands in Stata were used to adjust the clustering effect of the design.

The strength of the relationship between HBsAg carriage and vaccination status was assessed using Odds ratios (OR) (95% CI). A multivariable logistic regression model was built to control for the potential confounding effect of mother's serological status, age, and other potentially confounding variables.

2.8. Ethical aspects

The research protocol was approved by the Ethic committee medical ethics committee of the school of medicine, Universidad Nacional de Colombia, October 8, 2010. All participants were informed of the purpose and procedures of the study, and all of them gave a signed consent to participate. In addition, the children signed the required informed consent. Individuals positive for HBsAg marker were derived to their health management organization (HMO). A notification to the local health services was made to include the patients in the surveillance activities and monitor whether their take the appropriate steps to provide them health care and antiviral treatment.

3. Results

In total 1214 children were surveyed, of whom 42.1% lived in a rural area in Leticia, 24.9% in Tarapacá, 24.5% in Nariño and 8.5% in Puerto Santander. The proportion of female was 50.2% (610). The average age was 7.4 years (DE = 3.4 years) with minimum 1 month of age [25] and a maximum of 11 years old. A total of 46 (3.6%) samples from 1275 children were positive to the anti-HBc marker, of these, 7 (0.5%) were positive for both the anti-HBc and HBsAg. By age it is observed that in children younger than 4.5 years, no positive markers were found, in children from 4.6 to 7.3 years, 18 of the children had a positive marker for anti HBc and in those older than 10 years was in the group where the two children were found with DNA positive for HBV.

Children with timely vaccination (birth dose, and doses at 2, 4 and 6 months) had 70% less risk of being infected with HBV compared with those who did not (OR = 0.23, 95% CI 0.09–0.51). On the other hand, infants born to mothers HBsAg+ increased 2.5-fold the risk of being HBsAg carrier (OR = 2.45, 95% CI 1.33–4.46). Thirty-one anti-HBc+ samples (24 samples anti-HBc+/HBsAg− and 7 anti-HBc+/HBsAg+) were analyzed for the HBV genome. Two samples (2/31, 8.3%) were positive for the ORF S by PCR. The serological marker anti-HBc was detected in 176/572 (30.9%) (CI 95% 27.1–34.7) serum samples obtained from mothers, of these 52/572 (9%, CI 95% 6.4–11.1) were positive for both markers anti-HBc and HBsAg. Five anti-HBc+ serum samples were positive for the ORF S PCR (5/159, 3.1%) (110 samples anti-HBc+/HBsAg−, and 49 anti-HBc+/HBsAg+) (Table 2). All the positive blood samples were analyzed in duplicate.

In the children surveyed, a vaccination card was found to 93.0% (n = 1130). The children were vaccinated against hepatitis B in 90.8% of the cases, and only 22.8% (n = 277) had the age-appropriate vaccination against hepatitis B. The percentage of children who received a dose born for hepatitis B vaccine was 81.0% (n = 894), of these, only 34.1% (n = 305) had the dose before 48 h after birth. A total of 1059 children received the first dose of

Table 2
HBV infection markers in Amerindian children and mothers from the Amazonas.

Serological and molecular markers of HBV infection	Children n = 1275	Mothers n = 572
Anti-HBc+	46/1275 (3.6%)	177/572 (30.9%)
Anti-HBc+/HBsAg+	7/1275 (0.5%)	52/572 (9%)
Anti-HBc+/HBsAg−	38/1275 (2.9%)	124/572 (21.6%)
Viral genome detection (S region)	2/31 (6.4%)	5/159 (3.1%)
Anti-HBc+/HBsAg−/DNA HBV+	2/24 (8.3%)	0/110 (0%)

the pentavalent vaccine equivalent to 89.7%, of these 58.9% (n = 624), received this dose in a timely manner according to age. The second dose was administrated in 89.7% (n = 1059) of the children, and 51.0% (n = 620) had the vaccine according to age. The third dose of the pentavalent vaccine was applied to 86.9% (n = 1056), of which it was timely for age 41.0% (n = 434) (Table 3). Hepatitis B coverage based on the survey was higher than that reported by the Ministry of Health and Social Protection. In the survey areas, the highest hepatitis B coverage was in Leticia with 96.5% followed by Tarapacá with 92.7% (Table 3).

No differences were found by sex in children with hepatitis B infection (OR = 0.74, 95% CI 0.13–3.60, *p* Fisher = .494). Children younger than 10.2 years with HBV infection were no found. Infected children were found to the age group older than 10.3 years. There were also no differences in having received breast milk.

It was found that children vaccinated against hepatitis B presented a 99% lower risk of having been infected compared to unvaccinated children. This difference was statistically significant (OR = 0.01, 95% CI 0.00–0.10, Fisher P < .001) (Table 3). Children vaccinated opportunely against hepatitis B had no positive marker for the surface antigen (p = .1312) (Table 4).

Children with newborn doses against hepatitis B had a probability of 0.09 times of having the HBsAg positive marker than children without this dose and that difference is statistically significant (OR = 0.09, 95% CI 0.01–0, 46). Of the 7 children with a positive marker for HBsAg, only 2 had newborn doses after 48 h of birth (Table 4).

4. Discussion

Hepatitis B vaccination in the Colombian Amazon has proven to be highly successful according to the results of this study. Currently, less than 1% of children were found to be HBsAg carriers compared to 2% found in a study conducted in 1999, 8 years after vaccine introduction [14]. This further decrease in HBsAg carriage seems to reflect an important progress towards control of hepatitis B transmission among rural communities in the Colombian Amazon. In the Peruvian Amazon, a recent study by Cabezas et al., 2014 [4] has found a similar decrease in endemic levels of HBsAg carriage after universal HBV vaccination was introduced. Results from both studies suggest that the goal of <1% of carriers in children have been achieved in the Amazon.

On the other hand, mixed results have been reported from the Brazilian Amazon. Miranda Braga et al. [13] reported a lower HBV vaccination effectiveness, around 20%, and concomitantly a higher prevalence of HBsAg carriage after 19 years of HBV vaccination efforts [13]. However, other Brazilian researchers have informed a low prevalence of HBV infection in many other areas of the Brazilian Amazon. They found that HBV infection was only 1% of people born after HBV vaccine introduction [18]. Our results also remark the importance of increasing the efforts to improve the coverage with a timely birth dose in the Amazon's rural settings. It has been unclear what the role of perinatal transmission is in highly endemic areas of Latin America (LAC). In recent systematic review and meta-analysis by Ott et al., 2012 [19] it was found a lower prevalence of HBeAg among HBsAg childbearing-aged women in tropical LAC compared to prevalence in Asia Pacific (49% vs 53% at age 10–19 years; 36% vs 43% at age 20–29 years; and 27% vs 32% at age 30–39 years). We found that a late birth dose is related to a huge increase in the risk of becoming an HBsAg carrier, which suggests that perinatal transmission is important because horizontal transmission is now less so, due to the increment on the proportion of people exposed to the HBV vaccine. Observed HBsAg prevalence data are lacking in some regions and the quality of studies reporting these data is often low. Middle and low-income regions, e.g. Oceania, Central Asia, and Andean Latin America had a limited evidence base or studies were concentrated on one country as is the case with India as part of the South Asian region or Thailand located in the South East Asian region. To address the problems of representativeness, gray and non-English literature should be considered and studies on the prevalence of HBsAg were not available for the region.

However, an increasing the proportion of people receiving a timely birth dose may be challenging. Choconta Piraquive et al. [15] reported that the probability of receiving a timely birth dose is related to the chance of giving birth in a health facility, something that is difficult for many people living in rural areas of the Amazon. In Brazil, it has also been reported that compliance with birth dose is lower than expected even for urban populations [20].

In countries with high endemicity (prevalence of HBsAg $\geq$ 8%), HBV is transmitted mainly from mother to child at birth, or from child to child during childhood early (<5 years). In this type of epidemiological context, the application of vaccination schedules that administer the first dose of the vaccine at birth in this way, in more than 90% of the cases, women positive to HBsAg transmit HBV to their children. The vaccine should be given as soon as possible (<24 h) after birth. In this context, we found infection with hepatitis B virus alone in children older than 10.3 years; an indicator that is possibly related to a decrease in the transmission from mother to child. Other studies have shown the impact of vaccination on this transmission mechanism, which may support this finding [21–27].

Our study has several strengths. It is a population-based study with a low likelihood of selection bias since we achieved a remarkable high rate of participation; almost all households which were asked to participate accepted. Another source of selection bias may arise if unvaccinated HBsAg-positive children had more chance to be selected for the study than unvaccinated ones. This

Table 3
Vaccination coverage with hepatitis B vaccine found in children surveyed.

State	Vaccinated children	Children in surveys	Vaccination coverage	CI 95%
Puerto Nariño	245	296	82.8	78.1–86.7
Leticia	495	513	96.5	94.6–97.9
Tarapacá	279	301	92.7	89.3–95.2
Puerto Santander	84	104	80.6	72.3–87.5
Total	1.103	1.214	90.8	89.1–92.3

Table 4
Multivariate model for Anti-HBc+ and HBsAg+ serological markers of HBV infection in children from rural area of Amazonas State, Colombia.

Variable	Positive children for AntiHBc+/HBsAg+ marker+	+	–	OR crude	CI 95% (p value)	OR adjusted[a]	CI 95%
Sex	Female[c]	3	607	0.74	0.13–3.60 (P de Fisher = .494)	0.75	0.15–3.51 (p = .452)
	Male[b]	4	600				
	Total	7	1.207				
Age	Children over 10.3 years old[b,c]	7	296	1.31	0.27–7.00 (P Fisher = .370)	7.5	2.3–43.2 (p < .001)
	Children of other ages	0	911				
	Total	7	1.207				
Affiliation to the health system Colombia	Subsidized[b,c]	7	1.090	–	P Fisher = .99	–	p = .87
	Contributory	0	53				
	Not affiliated	0	42				
	Total	7	1.185				
Children vaccinated against hepatitis B	Yes[b,c]	1	1.102	0.01	0.00–0.10 P Fisher < .001	0.11	0.09–0.65 (p < .001)
	No	6	105				
	Total	7	1.207				
Children vaccinated against hepatitis B in a timely manner	Yes[b,c]	0	277	–	P Fisher = .131	0.06	0.002–0.21 (p < .001)
	No	7	819				
	Total	7	1.096				
Children vaccinated with doses against hepatitis B of newborn	Yes[b,c]	2	892	0.09	0.01–0.46 P Fisher < .001	0.10	0.03–0.24 (p < .001)
	No	5	204				
	Total	7	1.096				
Children vaccinated with doses against hepatitis B of newborn before 48 h	Yes[b,c]	0	305	–	P Fisher = .433	0.5	0.14–0.62 (p < .001)
	No	2	587				
	Total	2	258				
Variable	Positive children for AntiHBc+/HBsAg− marker	+	–	OR crude	CI 95% (p value)	OR adjusted[a]	CI 95%
Children in Tarapaca	Yes[b,c]	20	281	2.2	P Fisher = <.01	2.2	
	No	27	886				
	Total	47	1.167				
Children vaccinated against hepatitis B	Yes[b,c]	22	1.081	0.07	0.03–0.13 P Fisher < .001	0.11	0.09–0.65 (p < .001)
	No	25	86				
	Total	47	1.167				
Children vaccinated against hepatitis B in a timely manner	Yes[b,c]	7	270	0.50	0.208–1.106 P Fisher .064	0.06	0.002–0.21 (p < .001)
	No	40	786				
	Total	47	1.056				
Children vaccinated with doses against hepatitis B of newborn	Yes[b,c]	9	885	0.04	0.02–0.09 P Fisher < .001	0.01	0.001–0.24 (p < .001)
	No	38	171				
	Total	47	1.056				

[a] Logistic regression model.
[b] The reference variable is Positive children for AntiHBc+/HBsAg+ marker+ and all those that were coded with 1 and are marked with the symbol+.
[c] This symbol indicates the reference group for each variable.

type of selection bias is unlikely since participants were selected without knowledge of their serological or vaccination status.

There is also a low risk of information bias on vaccination status. Also, the vaccination status was ascertained using the child's vaccination card, thus reducing the risk of information bias on one of the main exposures. Furthermore, we looked for occult hepatitis B carriage using DNA-based methods which also improved the accuracy and validity of HBV infection outcomes.

Participants were selected using non-random sampling procedures and urban children were not included in the survey. This may preclude us to generalize the results to other populations in Colombian Amazon. Clearly, rural children have a higher risk of HBsAg carriage than urban children. Therefore, we may conclude that prevalence in children living in urban dwellings should have an even lower prevalence than what we presented here. However, non-random sampling procedures do not invalidate our findings on the protective effect of HBV vaccination and birth dose, which is perhaps the most important result in our study.

Even though the results of this study are encouraging, there is still a long path ahead in order to completely eliminate HBV transmission in the Amazon. HBsAg carriage is still high in people born before vaccination, 8% in mothers as showed in the result. This means that there is a large pool of people that can transmit HBV by horizontal mechanism, unprotected sex or others. Therefore, additional measures should be taken in the Amazon, like HBsAg carrier identification, follow-up, treatment and education on sanitary measures to reduce the risk of unadvertised HBV transmission.

In order to continue surveillance of seroprevalence in this region as well to consider the differences with Latin American studies, it is important to continue performing seroprevalence

studies that could include a standardized methodology every 10 year, to allowing the explanation of the findings on this study as well as the potential impact of hepatitis B vaccination in our region.

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