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Article/Artigo

Prevalence of hepatitis B virus infection and carriage after nineteen years of vaccination program in the Western Brazilian Amazon

Prevalência da infecção e de portadores do vírus da hepatite B, após 19 anos do programa de vacinação na Amazônia ocidental Brasileira

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ABSTRACT

Introduction: Reductions in the prevalence of hepatitis B virus (HBV) infection and carriage, decreases in liver cancer incidence, and changes in patterns of liver dysfunctions are described after hepatitis B vaccination. **Methods:** We conducted a population-based seroprevalence study aimed at estimating the HBV prevalence and risk of infection in the rural area of Lábrea following nineteen years of HBV vaccination. **Results:** Half of the subjects showed total anti-HBc of 52.1% (95% CI 49.6-54.7). The HBsAg prevalence was 6.2% (95% CI 5.1-7.6). Multivariate analysis showed an inverse association between HBV infection and vaccination (OR 0.62; 95% CI 0.44-0.87). HBsAg remained independently associated with past hepatitis (OR 2.44; 95% CI 1.52-3.89) and inversely to vaccination (OR 0.43; 95% CI 0.27-0.69). The prevalence of HBeAg among HBsAg-positive individuals was 20.4% (95% CI 12.8-30.1), with the positive subjects having a median age of 11 years (1-46) $p=0.0003$. **Conclusions:** We demonstrate that HBV infection is still an important public health issue and that HBV vaccination could have had better impact on HBV epidemiology. If we extrapolate these findings to other rural areas in the Brazilian Amazon, we can predict that the sources of chronic infected patients remain a challenge. Future studies are needed regarding clinical aspects, molecular epidemiology, surveillance of acute cases, and risk groups.

Keywords: HBV. Hepatitis B. Prevalence. Vaccination. Epidemiology.

RESUMO

Introdução: Reduções nas taxas de prevalência de infecção pelo vírus da hepatite B (VHB) e de portadores, incidência de câncer de fígado e mudança nos padrões de doenças hepáticas são descritos, depois da introdução da vacinação contra hepatite B. **Métodos:** Foi conduzido um estudo de soro prevalência de base populacional, com o objetivo de estimar a prevalência do VHB e fatores de risco de infecção na área rural de Lábrea, depois de 19 anos de introdução da vacinação contra hepatite B. **Resultados:** Metade dos indivíduos investigados mostrou reatividade ao anti-HBc total, 52,1% (IC 95% 49,6-54,7). A prevalência do HBsAg foi 6,2% (IC 95% 5,1-7,6). Análises multivariadas mostrou associação inversa da infecção pelo VHB e vacinação (OR 0,62; IC 95% 0,44-0,87). A presença do HBsAg permaneceu independentemente associada com o passado de hepatite (OR 2,44; IC 95% 1,52-3,89) e inversamente associado a história de vacinação (OR 0,43; IC 95% 0,27-0,69). A prevalência do HBeAg, entre os HBsAg positivos foi 20,4% (IC 95% 12,8-30,1), tendo em média os indivíduos positivos 11 anos de idade (1-46) $p=0,0003$. **Conclusões:** Foi demonstrado que o VHB é ainda um importante problema de saúde pública, e que a vacinação contra o VHB poderia ter tido um impacto maior na epidemiologia do VHB na região. Se esses achados forem extrapolados para outras regiões rurais da Amazônia brasileira, podemos prever que a fonte de pacientes crônicos é ainda um desafio a ser vencido. Estudos futuros devem focar os aspectos clínicos, a epidemiologia molecular, vigilância de casos agudos e grupos de risco.

Palavras-chaves: HBV. Hepatite B. Prevalência. Vacinação. Epidemiologia.

INTRODUCTION

Hepatitis B virus (HBV) infection has a global public health impact, with some 300 million chronic carriers worldwide¹. Clinical importance is associated with both fatal acute infection and chronic diseases (chronic hepatitis, liver cirrhosis, and liver cancer).

High prevalence rates have been described in Southeast Asia², Africa³, the Pacific Islands⁴, the Amazon region⁵⁻⁶, and Alaska⁷. In these areas, the virus is primarily transmitted perinatally from infected mothers, horizontally in early childhood⁸⁻¹⁰, or by blood exposure as a result of cultural practice¹¹.

In the Brazilian Amazon, prevalence rates of 70% for past infection and 15% for surface antigen (HBsAg) carriage have been described¹². Outbreaks of acute fatal hepatitis used to be very common in small rural villages, with a very high case fatality rate associated with hepatitis delta virus (HDV) infection of HBsAg carriers¹²⁻¹³.

Reductions in the prevalence of HBsAg infection and carriage, decreases in liver cancer incidence, and even changes in patterns of liver dysfunctions have been described after the implementation of hepatitis B vaccination programs in highly endemic countries¹⁴⁻¹⁷.

We conducted a population-based cross-sectional survey aimed at estimating the HBV serological marker prevalence and risk of infection in the rural zone of Lábrea county, Western Brazilian Amazon, following nineteen years of implementation of a hepatitis B vaccination program in the region.

METHODS

This is a population-based seroprevalence study of HBV infection. Lábrea is located in the Southwestern Brazilian Amazon (**Figure 1**), has an estimated rural population of 10,000, and was the first place in Brazil to receive the hepatitis B vaccine during the first national vaccination campaign in October 1989.

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FIGURE 1 - Map of the study area.

The region was first reached during the colonization period by those collecting forest products. In the nineteenth century, during the *rubber boom*, a huge number of *nordestinos*, those born in Northeast Brazil, resettled in the area to work at the natural rubber plantations. Currently, the villages are small, formed by those who remained after rubber extraction declined. The economy is still based on the exploitation of natural resources, such as seasonal agriculture at the half-year flooded banks of the Purus River, timber production, growing Brazil nuts, and raising livestock, with the products traded locally. The socioeconomic conditions are very poor, and the annual per capita income is around US\$1,000.

The target population consisted of all individuals living in the villages that compose the Lábrea County, along the Purus River. Villages were first randomly selected from a list from the Malaria Control Program, assuring that we had covered all the provincial geographical areas along the banks of the Purus River. Then, the research team randomly selected households in each village. The villages were visited from March 2005 to April 2006, during three major twenty-day expeditions and a few one-day trips to the villages near the town of Lábrea.

After signing the formal consent to participate, each individual filled out a questionnaire. A 10mL blood sample was taken from each of those present during the investigation; some family members were included later during our stay at the location because they were absent at the time of the visit.

The sample size was estimated using the official rural population census¹⁸, resulting in an estimated HBsAg prevalence of 6.4%¹⁹, a design factor of 2, $\pm 2\%$ precision, and 95% confidence level, which yielded a minimum sample of 1,009 individuals.

The serum samples were stored at -20°C locally before being flown to Manaus, where they were analyzed by enzyme-linked

immunosorbent assay (ELISA). All sera were tested for quantitative anti-HBs and total anti-HBc; those reactive to total anti-HBc and negative for anti-HBs were tested for HBsAg, and those found reactive were tested for HBeAg and anti-HBe using a commercial kit (DiaSorin S.p.A., Saluggia, Italy). The test procedures followed the manufacturer's recommendations and were performed automatically at the laboratory facilities of the virology unit of *Fundação de Medicina Tropical do Amazonas*.

Epi Info version 3.3.2²⁰ was used in data handling and sample size calculation; the explanatory variables included gender, age group, vaccination against hepatitis B, and personal antecedent of clinical hepatitis or jaundice. The study population was stratified by two-year groups for the first four years of life, then by 5-year increments up to the age of 19, and those 20 years or older, which comprised the population at risk of HBV infection before the implementation of hepatitis B vaccination.

The prevalence rates and corresponding 95% confidence intervals (95% CI) of HBV infection and carriage were estimated taking into account the study design. Logistic regression was used to calculate the crude ORs and 95% CIs. A final multiple logistic regression model including all study variables was used to control for confounding using Stata/IC 10.0²¹.

Ethical considerations

This study was reviewed and approved by the Research Ethics Committee of the Dr. Heitor Vieira Dourado Tropical Medicine Foundation (FMT-HVD), Manaus, Amazonas, Brazil (n°1775/2006 CEP/FMT).

RESULTS

This study included 1,510 individuals from 298 families selected in 54 visited villages. The infection and carriage rates varied significantly between villages, from as low as 0% in *Realeza* ($07^{\circ}35'52.8''\text{S}/66^{\circ}13'29.2''\text{O}$) to as high as 37.2% in *Madeirinho* ($07^{\circ}34'19.1''\text{S}/65^{\circ}26'31.4''\text{O}$).

Nevertheless, with regard to HBsAg prevalence, the great majority of the villages with positive subjects showed rates varying from high to moderate endemic patterns, as shown by the prevalence rates of 10.7% in *Limeira* ($07^{\circ}43'13.4''\text{S}/66^{\circ}42'33.7''\text{W}$) and 6.5% in *Praia do Buraco* ($07^{\circ}17'48.6''\text{S}/64^{\circ}58'28.3''\text{W}$).

Half of the sample (52.1%) showed serological markers of past HBV infection (95% CI 49.6-54.7). HBV infection was strongly associated with increasing age (Chi-square for linear trend=551.28, $p<0.001$) and positively associated with past history of clinical hepatitis (OR 2.31 (95% CI 1.74-3.06) $p<0.001$); it was also inversely related to HBV vaccination (OR 0.33 (95% CI 0.25-0.43) $p<0.001$) (**Table 1**).

The current infection (HBsAg+) prevalence was 6.2% (95% CI 5.1-7.6) and also increased significantly with age (Chi-square for linear trend=48.21, $p<0.001$). HBsAg had been present since very early ages, with 2% of those aged up to four years testing positive. However, the rates reached moderate patterns among adolescents (5.6%, 95% CI 4.31-6.89) and were as high as 10% (95% CI 9.34-10.9) within the cohort of those aged 20 years or more (**Table 2**).

HBV current infection also showed a positive statistical association with past history of clinical hepatitis (OR 3.17 (95% CI 2.04-4.90) $p<0.001$) and was inversely related to HBV vaccination (OR 0.33 (95% CI 0.21-0.56) $p<0.001$) (**Table 2**).

TABLE 1 - Hepatitis B infection (anti-HBc reactive) prevalence and associated variables, Rural Lábrea municipality, Western Amazon, Brazil, 2007.

Variable	Subjects (n)	Positive subjects			Crude OR (95% CI)	p-value	Adjusted	
		n	%	(95% CI)			OR (95% CI)*	p-value
Total sample	1,510	787	52.1	(49.6-54.7)	—	—	—	—
Age group								
≤2	136	24	17.6	(16.1-19.1)	1		1	<0.001**
3-4	114	14	12.3	(10.8-14.0)	0.65 (0.32-1.33)	0,24	0.73 (0.36-1.52)	
5-9	298	55	19.8	(18.8-20.8)	1.15 (0.68-1.94)	0,59	1.23 (0.72-2.12)	
10-14	216	84	38.9	(37.9-39.9)	2.97 (1.77-4.99)	<0,0001	3.11 (1.81-5.30)	
15-19	143	71	49.7	(48.5-50.9)	4.60 (2.66-7.97)	<0,0001	4.16 (2.35-7.35)	
≥20	603	535	88.7	(88.4-88.9)	36.7 (22.1-61.0)	<0,0001	33.6 (19.9-57.6)	
Gender								
female	749	389	51.9	(51.4-52.4)	1		1	0.87
male	761	398	52.3	(51.8-52.8)	1.01 (0.82-1.24)	0.88	0.98 (0.75-1.27)	
Past hepatitis								
no	1,184	587	49.6	(48.6-50.6)	1		1	
yes	268	186	69.4	(68.7-70.1)	2.31 (1.74-3.06)	<0.001	1.06 (0.75-1.49)	0.74
Hep B vaccine								
no	343	248	72.3	(71.7-72.9)	1		1	0.005
yes	1,113	514	46.2	(45.8-46.6)	0.33 (0.25-0.43)	<0.001	0.62 (0.44-0.87)	

Anti-HBc: antibody against hepatitis B core antigen; n: number; %: percentage; 95% CI: 95% confidence interval; Hep B vaccine: hepatitis B vaccine.

*Adjusted for the variables in Table 1; p-value: statistical significance; **Chi-square for linear trend; OR: odds ratio.

TABLE 2 - Hepatitis B surface antigen (HBsAg reactive) prevalence and associated variables, Rural Lábrea municipality, Western Amazon, Brazil, 2007.

Variable	Subjects (n)	Positive subjects			Crude OR (95% CI)	p-value	Adjusted	
		n	%	95% CI			OR (95% CI)*	p-value
Total sample	1,510	94	6.2	(5.2-7.6)	—	—	—	—
Age group								
≤2	136	3	2.2	(0.54-3.86)	1		1	
3-4	114	1	0.9	(-0.92-2.72)	0.39 (0.40-3.82)	0,42	0.47 (0.48-4.62)	<0.001**
5-9	298	12	4.0	(2.89-5.11)	1.86 (0.51-6.70)	0,34	1.83 (0.50-6.66)	
10-14	216	12	5.6	(4.31-6.89)	2.60 (0.72-9.41)	0,14	2.35 (0.64-8.61)	
15-19	143	5	3.5	(2.49-4.51)	1.60 (0.37-6.84)	0,52	1.21 (0.28-5.25)	
≥20	603	61	10.1	(9.34-10.9)	4.98 (1.54-16.14)	0,007	2.88 (0.87-9.55)	
Gender								
female	749	49	6.5	(5.8-7.2)	1		1	
male	761	45	5.9	(5.2-6.6)	0.89 (0.59-1.36)	0,61	0.81 (0.52-1.25)	0.35
Past hepatitis								
no	1,184	37	4.8	(4.3 - 5.4)	1		1	
yes	268	37	13.8	(12.7-14.9)	3.17 (2.04-4.90)	<0.001	2.44 (1.52-3.89)	<0.001
Hep B vaccine								
no	343	41	12.0	(11.0-13.0)	1		1	
yes	1,113	49	4.4	(3.8 - 4.9)	0.33 (0.21-0.56)	<0.001	0.43 (0.27-0.69)	<0.001

HBsAg: hepatitis B surface antigen; n: number; %: percentage; 95% CI: 95% confidence interval; Hep B vaccine: hepatitis B vaccine.

*Adjusted odds ratio for the variables in Table 2; p-value: statistical significance; **Chi-square for linear trend; OR: odds ratio.

After fitting a multiple logistic regression model including all the study variables, the association of HBV infection with past history of clinical hepatitis disappeared (OR 1.06 (95% CI 0.75-1.49) $p=0.74$). Nevertheless, we still detected an inverse association with HBV vaccination (OR 0.62 (95% CI 0.44-0.87) $p=0.005$) (Table 1).

Hepatitis B virus carriage remained independently associated with past history of hepatitis (OR 2.44 (95% CI 1.52-3.89) $p<0.001$) and inversely related to HBV vaccination (OR 0.43 (95% CI 0.27-0.69) $p<0.001$) (Table 2).

The prevalence of HBeAg among HBsAg-positive individuals was 20.4% (1993) (95% CI 12.8-30.1), with the positive subjects having a median age of 11 years (1-46) ($p=0.0003$) (Kruskal-Wallis test).

DISCUSSION

To the best of our knowledge, this is the first population-based seroprevalence study of HBV infection in the rural areas of the Western Brazilian Amazon, within the defined geographical limits,

conducted after the introduction of an immunization program, which allowed us to measure the impact of HBV vaccination in this single region.

We examined the HBV prevalence, taking into account the presence of two specific serological markers. Because the presence of total antibodies against the core antigen (total anti-HBc) is a marker of past infection, it can measure the importance of the circulation of the virus but lacks the strength for inferences concerning clinical aspects and transmission. Nevertheless, the presence of HBsAg can identify the transmission scenario and potentially chronically ill individuals.

While studies worldwide have found important drops in the rates of past HBV infection, as shown by a study in the Colombian Amazon, where less than 10% of the population aged 5-9 years and around 25% of those 10 to 14 years showed positive, representing a reduction of 72 and 62%, respectively, after the introduction of the vaccine²². In contrast, our study described much higher figures among the same age groups: 19.8% and 38.9%, respectively. In addition, we reported rates close to 50% in a group of individuals who should have been immunized after these nineteen years; that is, those aged 15 to 19 years, who belonged to the target population in the first five years of the introduction of the vaccine in the community.

Considering the rates of past infection reported before the introduction of the vaccine, which showed important variations (for example, from 15 to 32% for those up to five years old)²³⁻²⁴, the rate of less than 15% among those 3-4 years old reported in the present study suggests some impact of the vaccination program on new infections, especially among younger people. Still, we must be cautious with any conclusions because the majority of previous studies done in this region before the introduction of the HBV vaccine were analyses of non-probabilistic samples.

Our study was able to demonstrate that there has been an important circulation of the virus since very young ages among individuals who should have already been fully immunized. However, we may also stress the fact that the distribution of past infection was heterogeneous among the studied villages and that, in some villages, the whole studied population showed no contact with HBV.

Given that this heterogeneous pattern within villages has been described since the late 1960s⁵⁻⁶, HBV vaccination might have had little impact in changing the HBV epidemiology, which seems to have maintained its historical pattern. We believe that genetic factors²⁵ and the vaccination program strategies' failure to focus on risk groups may possibly explain some of these differences since the environmental and social economic conditions are very similar.

Unlike many other publications from former highly endemic countries describing a dramatic drop in the prevalence of HBV carriage after the introduction of the HBV vaccine in their national immunization programs^{17,26-28}, we report an overall prevalence of HBV carriage identified only in highly endemic areas, possibly with no counterpart in the literature thus far in the post-vaccination era.

Chronic carriers were found in all age groups. Indeed, we were able to distinguish three different cohorts with indications of important transmission intensity among those who theoretically should be protected. Infection among those aged up to two years supports the possibility of vertical transmission, while the prevalence among children aged between three and ten years indicates potential

horizontal transmission among young groups; within adolescents and young adults up to 19 years, it is possible that infection is due to sexual transmission. Since the virus circulates in all ages, the transmission is dependent on the type of risk factor present in each age cohort.

The prevalence and age distribution of HBeAg support the idea of important transmission among early ages because the presence of this serological marker indicates new infections, high viral load, and potential infectivity²⁹.

Taking into account that the prevalence of HBsAg carriage reported in this region before the introduction of the vaccine^{13,24} varied from 7% and 12%, there has been very little change in the epidemiological pattern of HBV distribution in this specific region from a hyperendemic situation to a highly to moderately endemic pattern. This is in contrast to the situation in Italy, where the prevalence of HBsAg dropped from 13% to less than 1% after twenty years of universal vaccination²⁶, and in Taiwan, which expected an overall prevalence rate of HBsAg carriage of less than 0.01% in the year 2010²⁷. In the Colombian Amazon, the prevalence of HBsAg carriage among those 5-9 years old dropped from 9% to 2%, indicating a reduction of more than 70% after eight years of HBV vaccination²².

We do not need to go too far to find better results of the HBV vaccination program in an Amazonian community. A study done in the year 2000 in the urban area of Lábrea not only demonstrated a low prevalence of HBV carriage among those born in the urban area and no carriers before adolescence but also suggested that the real problem with HBV infection would be in rural sites¹⁹.

Risk factors such as increasing age and past history of clinical hepatitis were strong predictors of HBV carriage even after those nineteen years, suggesting once again that the HBV vaccination program may have had little impact in changing the HBV epidemiology and on the aetiology of acute and chronic hepatitis in this region. Nevertheless, the hepatitis B vaccine has been shown to be an important protection against HBV, with those vaccinated having almost half the risk of infection and carriage of those not vaccinated, reinforcing the notion that the high figures we are describing may be related to a failure to deliver the vaccine to the population at risk.

In conclusion, our study demonstrates that HBV continues to be an important public health issue in the Amazon region and that the implementation of the HBV vaccination program in the rural area of Lábrea should have had a much stronger impact on HBV infection and carriage, as observed elsewhere. Vaccination strategies should focus on specific risk groups, such as infants born to a HBsAg-positive mother and adolescents. It seems that those who escape the vaccine intervention are responsible for keeping the prevalence rates of HBV infection and carriage among the highest still reported.

Hepatitis B vaccination programs are basically implemented to reduce HBsAg carriage because this marker of current infection is strongly correlated to chronic liver diseases, such as cirrhosis and liver cancer. The Amazonian environment may present operational difficulties for the delivery of any vaccine, mainly because of scarcity of resources and inadequate infrastructure. If we can extrapolate these findings to other rural areas in the Brazilian Western Amazon, we can predict that the sources of chronic infected patients remain a challenge. Future studies are needed regarding clinical aspects, molecular epidemiology, and surveillance of acute cases.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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