

Seroprevalence of Hepatitis B and C in the Western Brazilian Amazon Region (Rio Branco, Acre): A Pilot Study Carried Out During a Hepatitis B Vaccination Program

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In 1999, on the occasion of the application of the first vaccine dose during the state vaccination campaign against hepatitis B virus (HBV), 390 individuals from the town of Rio Branco, Acre, aged two or more years were selected for the determination of the seroprevalence of HBV and HCV. HBV markers (HBsAg, anti-HBs, and anti-HBc IgG) were determined on this occasion and anti-HBs antibodies were also assessed 30 days after the third vaccine dose. At the time of vaccination, 39% of the individuals were still susceptible to HBV, while 61% presented serologic evidence of previous HBV contact or previous vaccination. The individuals with previous HBV contact were significantly older ($p < 0.001$) than those without HBV markers. Of the 192 individuals who returned for reexamination, 30 days after the third dose, 158 (82.3%) had received three vaccine doses, and only 60 (31.2%) belonged to the group without HBV markers. In these individuals, the seroconversion rate after the third dose was 92% (55/60). In conclusion, we found considerable HBV in this population, indicating the need for pursuing the immunization programs. We also found high rates of vaccination coverage in the Western Brazilian Amazon region.

Key Words: Hepatitis B vaccination, Amazonia, hepatitis B, hepatitis C, epidemiology.

Hepatitis B is a worldwide public health problem, especially in developing countries. Chronic infection after exposure to hepatitis B virus (HBV) has been observed in 30% to 90% of children aged less than five years. On the other hand, exposed adults become chronic carriers of HBV in only 2 to 5% of cases [1]. In the United States, it is estimated that 500,000 to 1,000,000 individuals are chronically infected with the B virus, and 15% to 25% of them will die prematurely due to liver disease or hepatocellular carcinoma [2]. These aspects

of the natural history of HBV have prompted the implementation of vaccination programs against HBV in various countries worldwide [3-5].

In the Brazilian Amazon region, HBV infection is a severe health problem, which is complicated by the presence of hepatitis D virus (HDV) in the area. Fulminant hepatitis outbreaks due to HDV superinfection of HBV carriers is still frequently observed in this region [6]. No recent seroepidemiologic studies on HBV are available in the state of Acre, in the eastern Brazilian Amazon region, although evidence obtained from hospital reports and blood donor candidates indicates a high prevalence of individuals chronically infected with HBV. Most of them are adolescents or young adults, though older adults commonly suffer from acute hepatitis B.

Unfortunately, no population study concerning the prevalence of HCV is available so far. The large number

Received on 17 August 2003; revised 22 January 2004.

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The Brazilian Journal of Infectious Diseases 2004;8(2):133-139
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of HCV carriers in our outpatient unit seen at the referral hospital of Rio Branco (Acre) means that further prevalence studies are warranted.

The first-generation plasma-derived vaccine against the B virus was introduced in the 1970's [7]. In the 1980's, a recombinant HBsAg vaccine was produced, which showed excellent tolerance and efficacy [8-11].

In Brazil, the official immunization schedule includes the HBV vaccine, only for newborns and children younger than 15 years. Despite this recommendation, HBV vaccine coverage of newborns was less than 25% during the period from 1990 to 1998 in all 22 municipalities of the state of Acre (DABS - SESSACRE, unpublished data). This situation led the government of Acre, supported by the National Health Foundation and the National Immunization Program (PNI, Ministry of Health), to organize a mass vaccination campaign against HBV in 1996, which consisted of the application of three doses (at 0, 1 and 6 months).

Taking advantage of this ambitious vaccination program, we have studied serum HBV and HCV markers in a subset of vaccinated individuals from the town of Rio Branco, Acre, obtained before application of the first HBV vaccine dose and reexamined in part of the sample 30 days after the third dose.

Material and Methods

On the first day of the state vaccination campaign against HBV, seven mobile teams (each consisting of a medical student and a technician from the Central Public Health Laboratory of Acre) visited the 212 vaccination centers of the town of Rio Branco. Each team was responsible for one of the two district areas of the municipality of Rio Branco, an urban area with about 280,000 inhabitants. Rio Branco is the westernmost town among the large cities of the Brazilian Amazon region (Figure 1).

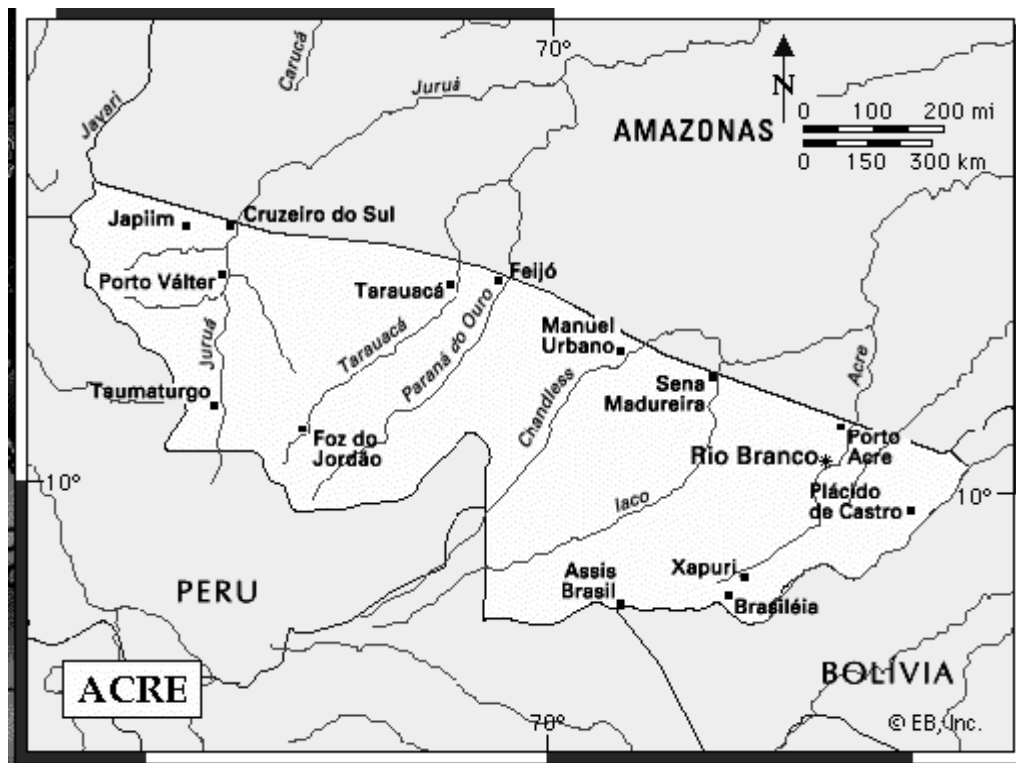
Upon the arrival of the mobile team at a given vaccination center, one or two individuals were selected for blood collection, answered a questionnaire and gave written informed consent to participate in the study.

The first individual selected was the person in the vaccination line who, upon arrival of the team, was seen by one of the volunteers ("vaccinator") of the respective center who recorded the personal data of each patient. At centers with more than 30 individuals per line, a second individual was selected, who was the person following the first one, provided that no family relationship existed between them. When one of these two persons refused to participate, the following person in the vaccination line was included. All infants aged less than 24 months were excluded due to difficulties in blood sample collection. A total of 394 individuals aged 2 to 79 years were evaluated.

The number of household dwellers and the number of rooms in the dwelling were obtained from the individuals included in the study (or their legally responsible guardian) as socioeconomic indicators. Patients reporting more than two individuals per room were considered to belong to a poor socioeconomic stratum, while two or fewer persons per room indicated a good socioeconomic level.

After filling out the questionnaire, 5 ml venous blood was collected without anticoagulant. The blood samples were then centrifuged and stored at -20°C at the Central Public Network Laboratory until they were sent to the Hepatitis Laboratory, Evandro Chagas Institute, Belém, Brazil, for the determination of serologic markers of HBV (HBsAg, anti-HBs and total anti-HBc IgG), HCV, and HDV. All markers were assayed using a commercial enzyme immunoassay (Organon®, Abbott, Chicago, IL, for HBsAg and anti-HDV; anti-HBs, anti-HBc IgG, and anti-HCV).

The second phase of the study was carried out one month after application of the third HBV vaccine dose between January 19 and January 21, 2000 (at this time, the serological results of the first serum samples were still unknown). Eight days before the beginning of the second phase all participants in the study (n=394) received a letter from the State Secretary Office of Health and Sanitation (SESSACRE), inviting them for a new blood collection for serological determination of anti-HBs markers. The reason for the study was explained in simple language accessible to the lay public. One hundred ninety two (49.2%) individuals accepted

Figure 1. Location of the State of Acre in the Amazon region

the invitation during the second phase and attended the Blood Center of Acre State (HEMOACRE). The blood samples obtained were then processed as described above, and the second module of the questionnaire was filled out.

The vaccine used during the state vaccination campaign against HBV was Engerix® B (SmithKline Beecham). The vaccine dose used was 10 µg for children aged 2 to 10 years and 20 µg for individuals 11 years or older.

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) software. Natives from the state of Acre and individuals born in the municipalities of the eastern region of the state were recorded as being from the Vale do Rio Branco region, while those born in municipalities of the central and western region were considered to be from Vale do Rio Juruá. The mean, standard deviation and median were determined for continuous variables, while

frequencies were assessed for categorical variables. The student's t test was used to analyse continuous variables and the X^2 test was performed to analyse categorical variables. The associations were considered to be statistically significant at an error probability of ≤ 0.05 ($\leq 5\%$).

Results

First phase of the study

Of the 394 individuals selected, four were excluded because of insufficient serum samples. The gender proportion was similar in this population ($n=390$; male: 48.2% vs. female: 51.2%), and mean age was 26.5 ± 14.9 years (median of 23 and mode of 13 years), range 2 to 79 years. The distribution of individuals according to positivity for the HBV serologic markers studied was

Table 1. Characteristics of the population from the Rio Branco municipality (Acre) according to hepatitis B virus (HBV) serologic group

Variable	Serologic group, n (%)				
	Total (n=390)	HBV carrier (n=13) ^a	Immune (n=81) ^b	Old inf. (n=144) ^c	Susceptible (n=152) ^d
Gender					
Female	202 (51.8)	6 (46.2)	43 (53.1)	71 (49.3)	82 (54.0)
Male	188 (48.2)	7 (53.8)	38 (46.9)	73 (50.7)	70 (46.0)
Age (years)					
Mean	26.5	35.7	15.4	36.7	21.9
± SD	14.9	13.2	9.9	14.1	11.2
Racial group					
Caucasian	183 (46.9)	7 (53.8)	36 (44.4)	70 (48.6)	70 (46.0)
Non-Caucasian	207 (53.1)	6 (46.2)	45 (55.6)	74 (51.4)	82 (54.0)
Origin					
Acre	316 (81.0)	10 (76.9)	71 (87.6)	115 (79.9)	120 (79.0)
Other states	74 (19.0)	3 (23.1)	10 (12.4)	29 (20.1)	32 (21.0)
Acre region^e					
Rio Acre	257 (81.3)	7 (70.0)	68 (95.8)	75 (65.2)	107 (89.2)
Rio Juruá	59 (18.7)	3 (30.0)	3 (4.2)	40 (34.8)	13 (10.8)
History of vaccination					
No	291 (74.6)	8 (61.5)	43 (53.1)	109 (75.7)	131 (86.2)
Yes	99 (25.4)	5 (38.5)	38 (46.9)	35 (24.3)	21 (13.8)
History of hepatitis					
No	337 (86.4)	9 (69.2)	71 (87.6)	127 (88.2)	130 (85.5)
Yes	53 (13.6)	4 (30.8)	10 (12.3)	17 (11.8)	22 (14.5)

(a) HBV carrier (HBsAg positive and anti-HBc positive); (b) Vaccinated or immune (anti-HBs positive and negative for the other markers); (c) Past infection (anti-HBc positive, HbsAg negative and anti-HBs positive or negative); (d) Susceptible (negative for all three markers); (e) Including 316 individuals from the Vale do Rio Acre and Vale do Rio Juruá regions.

as follows: 3.3% (n=13) were positive for HbsAg and 34.3% (n=134) for anti-HBs and anti-HBc IgG. Anti-HBc IgG alone was detected in 13 (3.3%) individuals. Anti-HBs alone (previous vaccination) were observed in 14.1% (n=55) of the individuals. Only 39.0% (n=152) of the population studied was seronegative for all three HBV serologic markers (HBsAg, anti-HBc and anti-HBs). Table I shows the distribution of the serologic groups, i.e., previously vaccinated, previous HBV exposure, and two patients with HDV co-infection.

Of 386 (99.0%) individuals studied, 16 (4.2%) were positive for anti-HCV, but no confirmatory test was applied. Table 1 also shows the demographic characteristics of the sample, with a similar gender distribution ($p>0.48$) being observed for each serological group. Mean age was significantly higher in the HBsAg-positive group (35.7 ± 13.2 years) and in the group with HBV exposure (36.7 ± 14.1 years; $p<0.03$ and $p<0.001$, respectively) and was significantly lower in the groups of previously vaccinated individuals (15.4 ± 9.9 years) and individuals without HBV markers (21.9 ± 11.2 years; $p<0.0001$).

Race distribution (whites = 183, non-whites = 207; $p > 0.59$) and origin (born in the state of Acre vs. born in other states; $p > 0.39$) were similar among the serological groups. When individuals from the state of Acre ($n = 316$, 81.0%) were analyzed according to the region of the municipality where they were born (Vale do Rio Acre or Vale do Rio Juruá), individuals from the towns of Vale do Rio Juruá were significantly more frequent in the group with a past infection ($p < 0.001$) and less frequent in the group of vaccinated ($p < 0.001$) or susceptible individuals ($p < 0.006$).

A strong association with previous immunization ($p < 0.001$) was observed in individuals positive for anti-HBs, without anti-HBc IgG ($p < 0.001$, Table 1). However, a similar distribution of the vaccination history was observed for HbsAg-positive individuals ($p > 0.20$), and in those with a past and resolved infection ($p = 0.69$). The history of jaundice was similar for the serological groups studied (HBsAg, $p > 0.08$; anti-HBs positive with and without anti-HBc IgG, $p > 0.70$; individuals without HBV markers, $p > 0.70$).

In the analysis of the distribution of individuals according to the number of rooms in the dwelling, most of them (64.9%, 253/390) belonged to the stratum of ≤ 2 persons/room, while the distribution of those with more than two persons/room was similar for the serological groups.

Second phase of the study

Only 192 (49.2%) of the 394 invited individuals participated in the second phase of the study, with return of the letters to the sender (SESSACRE). The main reason (108/198, 54.5%) for the lack of participation was related to mail and address changes.

Among the 192 individuals analyzed, 158 (82.3%) reported the use of three HBV vaccine doses, 28 (14.6%) received two doses and 6 (3.1%) only one dose on the occasion of the state vaccination campaign against HBV. Forty-five (23.4%) of them were previously immune (anti-HBs positive, detected on the occasion of the administration of the first vaccine dose), 8 (4.2%) were HBV carriers (HbsAg positive), and 79 (41.2%) presented a serology compatible with a

past and resolved HBV infection, i.e., only 60 (31.2%) individuals were still susceptible to HBV (HBsAg, anti-HBs and anti-HBc negative).

Among the 60 individuals susceptible to HBV, most ($n = 50$, 83%) received three vaccine doses, 7 (12%) received two doses, and 3 (5%) only one dose, with the anti-HBs seroconversion rate (serum sample obtained 30 days after the third vaccine dose) being 92% (46/50), 100% (7/7) and 67% (2/3), respectively.

Of the 5 (8%) non-seroconverted cases (anti-HBs negative 30 days after the third vaccine dose), one had received only one dose and four had received three doses.

At the end of the study, the results of the serological tests (including those positive for anti-HCV) were sent to the 390 individuals by regular mail. When appropriate, the person was advised to attend the Service of Infectious and Parasitic Diseases of the General Hospital of Rio Branco. Community health agents from the area in question tried to identify cases of previously returned correspondence ($n = 108$) or those returned on the second occasion ($n = 41$), and were able to locate 136 (91.3%) individuals.

Discussion

Second-generation vaccines using recombinant viral protein have provided efficient prophylaxis against HBV, but despite these efforts about 350 million people are estimated to be infected with the virus, rendering HBV an important carcinogen [12]. Nevertheless, vaccines and other control measures have led to a decline in the prevalence of HBV over the last few years, although HBV infection continues to be a severe public health problem worldwide, with some regions such as Asia, the east coast of Africa and the Amazon region being hyperendemic [13]. Especially in this region of Latin America, vaccination against HBV can also lead to an associated decline in the prevalence of HDV.

In this population, although vaccination of children younger than 15 years, including newborns, has been encouraged by health authorities, only 25.4% of the

individuals reported previous vaccination against HBV, a rate similar to the vaccination frequencies reported for the 22 municipalities of the state of Acre during the period from 1990 to 1998 (DABS - SESSACRE, unpublished data).

The number of cases in the state of Acre with chronic viral hepatitis or cirrhosis, including patients younger than 25 years and individuals transferred for treatment outside the home (DCA - SESSACRE, unpublished data), demonstrates the relevance of HBV as a health problem, and also explains the great mobilization of the population on the days of state vaccination, as well as the high rates (>82%) of vaccination coverage reached on the days of the state campaign (DABS/PNI - SESSACRE, unpublished data).

As an example of the relevance of HBV infection in the state of Acre, even though acute hepatitis B caused jaundice in only one-third of the cases [14], a history of hepatitis was reported by 15.7% of the individuals studied. In contrast, a large part of the population tested positive for HBsAg (3.3%) or for HBV serologic markers compatible with past exposure. Another group of individuals (20.8%) presented isolated anti-HBs compatible with a history of vaccination, a frequency similar to that observed for individuals who reported previous vaccination (25.4%). Only 39.0% of the vaccinated individuals were still susceptible to HBV infection (HBsAg, anti-HBs and anti-HBc negative) and thus benefitted from the vaccination program. Therefore, it is important that in this region vaccination against HBV is preceded by serum anti-HBs determination in isolated cases or in small population groups, an unfeasible and expensive step when applied to vaccination programs similar to that carried out in the state of Acre.

In addition, the rejection rate of blood donor candidates due to anti-HBc seropositivity has been found to be abnormally high in the Amazon region when compared to other regions of the country [15]. This is further demonstrated by the previous observation that 36.9% of individuals presented HBV markers.

Recently, further concern has been raised regarding the high prevalence of anti-HCV among blood donor candidates in this region. This fact, together with the

exclusion of blood donors due to anti-HBc IgG seropositivity, has generated a serious shortage of blood in the region.

Despite the pilot study character of the present investigation, these indicators, in addition to the high prevalence of anti-HCV (4.2%) and anti-HDV (0.5%), demonstrate the relevance of hepatotropic viral infections in this region of the country [6] and should thus encourage further action by government and research institutions.

Unfortunately, no confirmatory test was performed in order to validate ELISA anti-HCV-positive cases. Nevertheless, we found a high prevalence of anti-HCV in this population, which seems to be very close to the actual prevalence of HCV in blood donor candidates from the state of Acre. In addition, the serum samples were handled under appropriate conditions and stored at -20°C, as soon as collected.

Although associated with or influenced by multiple biological, social and economic factors in Brazil, gender and race distribution was similar among the four serological groups, further supporting the assumption of homogeneity of the sample and the wide dissemination of HBV in the population, irrespective of other social and biological characteristics. In this respect, the similarity between the serological groups in the proportion of individuals living in dwellings with more than two persons/room also demonstrates that the circulation of HBV in the population from Rio Branco town seems to be independent of socioeconomic level.

No difference was observed between individuals born in the state of Acre and those from other states of the country, although this type of association is affected by multiple variables, including the time of residence in the Amazon region or, more specifically, in the state of Acre.

The impact of HBV infection has been known to be more relevant among residents in the Vale do Rio Juruá municipality (DABS - SESSACRE and HEMOACRE, unpublished data), in agreement with the significantly higher frequency (67.8%, $p < 0.001$) of anti-HBc-positive cases among individuals from this area.

The high frequency (82.3%) of individuals receiving three vaccine doses among those reassessed 30 days

after the third dose demonstrates good compliance of the population with the state vaccination campaign.

Although one may speculate about the large number of individuals who did not respond to the call during the second phase, this apparently did not cause distortions in the results, since a similar distribution of the variables was observed for individuals who participated in the second phase of the study (n=192) and those studied during the first phase (n=390). The few non-seroconverted cases (n=5), especially the four individuals who received three vaccine doses, might be explained by factors associated with vaccine application, the vaccine itself or host characteristics [16,17].

In conclusion, evidence of the strong impact of HBV on public health demonstrates that a similar vaccination program could be applied in places with characteristics similar to those of Acre state, where large, geographically isolated areas prevent the implementation of various public programs or services. This study also demonstrated that HCV infection is an important problem in the Brazilian Amazon region, which requires further studies and greater attention on the part of federal government health authorities.

Acknowledgments

We thank the medical students of Acre and the students of the Medical School of Cuba, as well as Master's student Jailson de Araújo Silva (UFBA) and the students Juliano Freitas-Andrade (UFBA), Ciro Falcão Macedo Jr. (UNI - RJ), and Thiago Neves Paiva (UBM - RP, SP). This study was supported by SESSACRE (Government of Acre), CNPq, and the CAPES/COFECUB 404/02 project as part of the French/Brazilian Study Group on Viral Hepatitis.

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