

## Serological Markers of Hepatitis A, B and C Viruses in Rural Communities of the Semiarid Brazilian Northeast

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In the village of Cavunge, located in a dry tropical, semiarid rural region of the state of Bahia, Brazil, a sentinel study on viral hepatitis is underway. We report on the first part of the study. The objective of this study was to determine the prevalence of serological markers for hepatitis A, B and C in the village. Cross sectional study. Blood samples were tested for serological markers of hepatitis A (HAV), B (HBV) and C (HCV) through ELISA-III assay. In HBsAg and anti-HCV carriers, HCV-RNA and HBV-DNA were checked by PCR. The prevalence of anti-HAV IgG was 83.3% (1,210/1,452), being higher among residents from the village (87.4%) than in residents from the rural area (79.5%); it also higher among individuals older than 10 years of age. The prevalence of HBsAg was 2.6% (38/1,476), 9.3% anti-HBc (137/1,476) and 10.5% (155/1,476) anti-HBs of. In more than half (58.1%; 90/155) of anti-HBs carriers, this was the only serological marker found. In 3.7% of the population, (55/1,476), anti-HBc was the only serological marker found. All HBV carriers were infected by genotype A. Only 0.4% (6/1,536) presented anti-HCV antibodies and only one of them was viremic, being infected with genotype 1. The prevalence of patients with antibodies against hepatitis A virus in the village of Cavunge was high, but the prevalence of B virus was moderate, with only genotype A among HBV carriers. The prevalence of C virus was very low, contrasting with the situation in large Brazilian urban centers.

**Key Words:** Hepatitis A, hepatitis B, hepatitis C, epidemiology, Brazil.

Hepatitis A, B and C viruses have distinct distributions; their epidemiological particularities should be studied, to determine ethnic, genetic, environmental and cultural aspects of each population. In Brazil, due to the existence of several distinct ecosystems, seroprevalence studies have shown a wide variability of the distribution of hepatitis B, C and D virus carriers [1-3]. Similarly, there are differences in the prevalence of the antibody against HAV [4].

In the village of Cavunge, district of Ipacaetá (state of Bahia), the demographic and socioeconomic characteristics are typical of this scrub savanna region, with a dry tropical climate and low socio-economic levels [5]. This region is completely different from the Amazon region, where the climate is humid, with a large variety of flora and fauna [6].

The prevalence of hepatotropic viruses in the Brazilian northeastern semiarid region, where the village of Cavunge is located, should be better evaluated, since few population based studies have been made in that region [2,7]. In contrast, there is a high prevalence of hepatotropic viruses in the Amazon region with virological peculiarities, such as genotype F of HBV [8,9] and hepatitis D virus [10]. Many Brazilian

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studies, as well as international studies, describe the prevalence of hepatotropic viruses among specific groups, such as blood donors [11], patients in hemodialysis programs [12-14], hemophiliacs [15], afro-descendants [16], mental disease patients [17] and hepatitis patients [18,19]. Due to a lack of studies conducted on the Brazilian northeastern semiarid region, we examined the distribution of hepatitis A (HAV), B (HBV) and C (HCV) serological markers in this population.

### Material and Methods

#### Characteristics of the region

The village of Cavunge belongs to the district of Ipacaetá (state of Bahia), 162 km from Salvador, the state capital. Its has an area of approximately 63.5 km<sup>2</sup>, with a recorded population in 1999 of 2,049 individuals, with ages ranging from seven days to 95 years. The economy of the region is based on subsistence agriculture (beans, corn and cassava); cattle raising is almost always restricted to large farms [5].

#### Blood collection and clinical evaluation

In 2003, serological markers of hepatitis A, B and C were determined for all serum samples collected in 1999 from the population of Cavunge (village and surrounding farm lands). These samples were part of a previous study on the oligosymptomatic form of visceral leishmaniasis. After organizing the databank and the sera, a domiciliary visiting

program was organized with the participation of the Family Health Unit Program (FHUP). This included from the population of Cavunge those who agreed to participate in the study by signing the post-informed consent form and who responded to the socio-demographic and epidemiological questionnaires. In this phase, only those who met the inclusion criteria were included in the study (fixed residence more than six months in the village of Cavunge). Patients with chronic-degenerative mental disease (unable to understand the objectives of the study), regardless of age and as long as they had no legal guardian, were excluded. The project was approved by the Ethics Committee in Research of the University Hospital and also by the National Ethics Research Council (CONEP).

Next, a domiciliary visiting program was organized in order to visit individuals seropositive for serological markers of hepatitis B and C and to schedule visits to FHUP for clinical evaluation and new blood collections. Seropositive individuals responded to a questionnaire that evaluated possible risk factors associated with hepatotropic viroses, including: previous history of surgery, blood transfusion, tattooing, piercing, use of intravenous drugs, contact with relatives infected with hepatotropic viruses, sexual activity, use of condoms, number of sexual partners, history of sexually-transmissible infections, shared use of personal objects, history of alcoholism, among others. The physical examination was complete, with special attention to alterations compatible with chronic liver parenchyma disease.

#### Serological examinations

Serological markers of hepatitis A, B and C viruses were examined in the Clinical Pathology Laboratory of the Aliança Hospital (Salvador - Bahia) and the Bahia Central Laboratory (LACEN) using the ELISA-III assay (Roche, Basel-Switzerland). Clinical laboratory tests were performed using the dry chemical technique (Johnson, USA).

The serological markers that were examined were: anti-HAV IgG, HBsAg, anti-HBc IgG, anti-HBs and anti-HCV. Whenever HBsAg or anti-HCV was positive, the sera were sent to the Gonçalo Moniz Research Center (CPqGM – FIOCRUZ, Salvador), in co-operation with Unit 271, INSERM, Claude Bernard University, Lyon, France for the HBV-DNA and HCV-RNA through the PCR ultra-sensitive technique.

The ultra-sensitive PCR technique for HBV-DNA detection consists of amplifications in two stages (nested or semi-nested PCR), using primers located at well-conserved regions of the viral genome. The PCR technique was performed with one fifth of the total nucleic acids extracted from 140µl of serum (Qiagen), based on Chemin et al. (2001) [20]. Genotype determination was performed through INNOLIPA (Imunogenetic, Belgium). The HCV-RNA exam was performed through RT-PCR, as described by Silva et al. (2000) [2].

Aminotransferases were also determined for individuals positive for hepatitis B or C. Whenever indicated, a complete

laboratory hepatic evaluation was made (total protein, albumin, globulin, ferritin, bilirubins, alkaline phosphatase and alpha-feto protein), along with medical imaging evaluation (abdominal ultrasound and upper digestive endoscopy).

#### Statistical analysis

The statistical analyses were performed with the aid of SPSS ("Statistical Package for Social Science" v. 9.0 for Windows, SPSS Inc, Chicago, Illinois) software. For the continuous variables, the mean, median and standard deviation were determined. The categorical variables were expressed using the absolute numbers and the proportions of each variable.

#### Results

For 460 individuals (22.4% of the total population, n=2,049), blood collection could not be made due to absence at the dwelling or due to refusal. Hemolysis in the serum sample was observed for 24 (1.2%) individuals. Therefore, 1,790 serum samples collected in 1999 remained,; but some of them were not sufficient to do serum research for all viral markers. The demographic data of the population are found in Table 1. Almost half of the individuals (44.6%, n= 799) were illiterate, and the main occupation of the head of the family was agricultural work (48.8%, n= 874); 84.6% of the population was composed of African-descendent individuals, which is characteristic of this Brazilian region.

**Table 1.** Demographic characteristics of the Cavunge, Bahia population, 1999

Variable	N (%)
<b>Localização</b>	
Rural	855 (47.8)
Urban	935 (52.2)
<b>Gender</b>	
Male	876 (48.9)
Female	914 (51.1)
<b>Race*</b>	
White	244 (14.8)
Mulattos	1,136 (68.8)
Afro-descendants	261 (15.8)
Amerindians	9 (0.5)
Albinos	1 (0.1)
<b>Age</b>	
Mean ± SD	30.3 ± 23.1
Median	23.5
Limits	0 – 149
<b>Leader's family time of residence</b>	
Mean ± SD	31.9 ± 22.7
Median	30.0
Limits	0 – 93

\* Race was not reported by 139 individuals.

Table 2 shows the number of serum samples researched for each marker and the respective number of positives.

**Table 2.** Serological hepatitis markers studied in the Cavunge, Bahia population, 1999

Serological markers	N tested	Positives – N (%)
Anti-HAV	1,452	1,210 (83.3)
AgHBs	1,476	38 (2.6)
Anti-HBc	1,548	151 (9.8)
+ AgHBs	1,468	12 (0.8)
+ Anti-HBs	1,368	65 (4.9)
Anti-HBs isolated	1,331	90 (6.8)
Anti-HCV	1,536	6 (0.4)

#### Hepatitis A virus (HAV)

The prevalence of anti-HAV IgG antibody carriers (Table 2) in Cavunge was 83.3% (1,210/1,452). Seropositive individuals were more frequent ( $P < 0.001$ ) among residents in the village (87.4%;  $n = 618$ ) than in those who lived on farms (79.5%;  $n = 592$ ). The distribution of seropositive individuals was similar ( $P = 0.095$ ) among men (81.6%;  $n = 559$ ) and women (84.9%;  $n = 651$ ).

The anti-HAV (IgG) seropositivity increased proportionally ( $P < 0.001$ ) with age (Table 3), and the average age of the seropositive individuals ( $34.7 \pm 22.0$  years) was significantly higher ( $P < 0.001$ ) than for the seronegative individuals ( $9.1 \pm 10.9$  years).

**Table 3.** Serological prevalence of hepatitis A virus (HAV) in Cavunge, Bahia by age interval

Age intervals	Individuals	Anti-HAV positive <sup>§</sup>	
		N	%
0-10	289	117	40.5
11-20	367	312	85.0
21-30	178	170	95.5
31-40	160	158	98.8
Upper 40	443	438	98.9
<b>Total</b>	<b>1,437*</b>	<b>1,195*</b>	<b>83.2*</b>

\* In 15 individuals age was not registered and 100% of them were anti-HAV positive. §  $P < 0.001$ .

The 1,452 individuals lived in 434 dwellings. We were able to analyze all residents of 240 dwellings. In only one of these dwellings (0.4%), all individuals were seronegative and in 156 (65.0%), all individuals were seropositive.

#### Hepatitis B virus (HBV)

The prevalence of individuals seropositive for HBsAg and anti-HBc IgG was 2.6% (38/1,476). The demographic characteristics of these individuals are described in Table 4. Table 2 shows the seropositive frequency for the other HBV

markers. Anti-HBs was positive in 11.6% (155/1,331), and in more than half (58.1%, 90/155), this was the only serological marker found. The average age of the individuals with isolated positive anti-HBs was 10.22 years ( $\pm 15.7$ ). Within this group, 78.9% (71/90) were not older than seven years. Among the total population, 3.7% (55/1,476) of the individuals presented anti-HBc IgG as the only positive serological marker.

**Table 4.** Demographic profile of individuals by AgHBs status

Variable	AgHBs positive	AgHBs negative	P
<b>Residence – N (%)</b>			0.005
Urban	9 (23.7)	675 (46.9)	
Rural	29 (76.3)	763 (53.1)	
<b>Gender – N (%)</b>			0.043
Male	24 (63.2)	669 (46.5)	
Female	14 (36.8)	769 (53.5)	
<b>Age – yrs <math>\pm</math> SD</b>	30.8 $\pm$ 22.9	34.7 $\pm$ 22.9	NS*
<b>Race – N (%)</b>			0.034
White	1 (2.6)	217 (15.1)	
Non-white	37 (97.4)	1,216 (84.9)	

\*NS= Not significant.

Among HBsAg carriers, only one 24-year-old individual with daily ethanol consumption of 80 g/day during the previous five years presented signs of chronic liver disease, with portal hypertension and esophageal varices development. The other HBsAg carriers presented normal serum aminotransferase dosages and normal clinical exams, which is compatible with an inactive carrier state.

HBV-DNA was positive in 9 (23.6%) out of the 38 cases, and all presented genotype A. Among these, four male individuals belonged to the same family (two brothers, one of them with two children also infected). In this family, among the risk factors for HBV infection, shared use of razor blades was reported.

#### Hepatitis C virus (HCV)

Among samples analyzed for anti-HCV at the initial phase of the study, only 0.4% (6/1,536) presented a positive result (ELISA). Based on HCV-RNA, only one patient (0.06%) presented viremia, but no alterations in aminotransferase levels were observed in four repeat exams. Viral genotyping revealed subtype 1. This individual had lived for more than 10 years in a large urban center and did not present previous surgical intervention or other risk factors associated with HCV infection.

#### Discussion

In Brazil, knowledge on hepatitis A, B and C still presents gaps, especially in the Brazilian northeastern region, where

population prevalence studies among rural populations are scarce [7]. Several factors observed in Brazil involving geographic region, socioeconomic situation, racial and cultural differences, among others, lead us to believe that there are significant differences in the prevalence of these viruses.

In the state of Bahia, prevalence studies on HBV and HCV virus are almost always restricted to blood donor candidates in the city of Salvador and among individuals in hemodialysis programs [12]. A single population study suggested that HCV prevalence is higher in urban than in the rural regions [2].

The seroprevalence of individuals with antibodies against HAV observed in our study (83.3%) was similar to that found in other regions of the country, where frequencies are also high [21], especially in areas with low social and economic development indexes [22].

A significant increase in seroprevalence of anti-HAV carriers was proportional to increase in age. In semiarid northeastern locations, especially smaller towns such as the village of Cavunge, environmental pollution is probably the main determinant of several fecal-oral transmission diseases. In this context, this population is different from other more-developed regions of the country, where HAV infection is observed in older individuals [23].

The HBsAg prevalence found (2.6%) places this region among the intermediate endemicity regions for HBV. This makes sense given the low frequency (9.3%) of anti-HBs carriers, which is also due to the low vaccination demand. Surprisingly, the epidemiological surveillance program does not consider this area at high risk for HBV, and the establishment of a universal vaccination program against HBV has been delayed. Isolated anti-HBs were found mostly in children, suggesting that the vaccination program against HBV has recently been reinforced by the local FHUP.

Our data confirm the observation that HBV genotype A largely prevails in this Brazilian region. This population has little migratory influx. Thus we suppose that HBV genotype A was introduced in this population a long time ago and remained as the most prevalent genotype in this region.

The very low frequency of HCV carriers (0.4%) observed for the population of Cavunge not only corroborates the findings of Tavares-Neto et al. (2005) [7], but also seems to be typical of rural areas in northeastern Brazil.

The large extension of the Brazilian territory and the variability of its ecosystems and population, especially with regard to socioeconomic indicators, justify a need for further studies in other northeastern Brazilian regions as an information tool for planning improvements and follow-up of programs aimed at improving public health.

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