Epidemiology and Molecular Characterization of Hepatitis B Virus Infection in Isolated Villages in the Western Brazilian Amazon

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Abstract. Individuals from three isolated, rural communities in the western Brazilian Amazon were evaluated for serological markers of hepatitis B virus (HBV) infection, HBV genotype, and the presence of risk factors for infection and transmission. Of the 225 individuals studied, 79.1% had serological evidence of HBV infection; 10.2% individuals were chronic carriers for HBV surface antigen (HBsAg-positive). Analysis of risk factors indicates that HBV is transmitted mainly horizontally within the family from a chronic “active” carrier for hepatitis B “e” antigen (HBeAg-positive), though a strong possibility of vertical transmission remains. The predominance of HBV genotype F, with a higher genomic similarity between the isolates, indicated a relatively recent introduction of HBV, from a common source, to the area. This study sheds light on the HBV epidemiology in the Brazilian Amazon region and highlights the need for greater emphasis on HBV control and immunization programs.

INTRODUCTION

Hepatitis B virus (HBV), a significant threat to public health, is one of the most important human pathogens. Approximately two billion people worldwide present serological evidence of past or current HBV infection and nearly 360 million individuals are estimated to be chronic carriers of HBV that may progress to death from cirrhosis and its complications, and hepatocellular carcinoma.1

The Amazon is one of the regions with a high prevalence of diseases associated with HBV infection.2–4 Since the early 1960s, outbreaks of fulminant hepatitis associated with hepatitis D virus (HDV) superinfection of an HBsAg carrier, known as “Lábrea Black Fever,”5 have been reported from rural communities of the region. Despite its burden on the health of the population, the mechanisms of transmission of HBV in the Amazon are still not clearly defined. Vertical transmission does not appear to be significant, but intra-family transmission, associated with the presence of an HBV carrier is commonly described.6,7

The HBV is classified into 10 genotypes (A to J), originally distributed within specific populations. Human migrations and miscegenation defined the pattern of geographical distribution observed today.3,8–10 Genetic diversity, besides being associated with the clinical severity, treatment failure, and factors affecting vaccine response,11 may also contribute as tools for characterizing transmission patterns.12–14

Seroepidemiological studies, carried out in the municipality of Lábrea after the introduction of the hepatitis B vaccine in 1989, revealed a pattern of low and moderate endemicity of HBV infection with an anti-HBc total prevalence of 27.9% among those born in the city of Lábrea to a high prevalence among individuals from the rural zone (67.9%).12 In view of these results, 54 rural communities along the Purus River, at the outer edges of the municipality, were studied and a heterogeneous pattern of HBsAg prevalence was found, ranging from 0% to 37.2% among the communities evaluated.15

The aim of this study was to determine the HBV prevalence, risk factors associated with HBV transmission in three communities previously screened, and the phylogenetic analysis of the HBV. The communities were chosen based on the rate of HBV infection in the municipality of Lábrea, a region in the Purus river basin of the Amazonas state of Brazil.

MATERIAL AND METHODS

All individuals of the three riverines communities of the municipality of Lábrea were evaluated: Madeirinho (07°34′19.1″S/65°26′31.4″O); Praia do Buraco (07°17′48.6″S/64°58′28.3″W), and Samauima (07°18′51.1″S/65°08′42.1″W) (Figure 1). As the transmission dynamic was being studied, the three communities were chosen based on their increasing endemicity of HBsAg.15

The families were evaluated in their homes after obtaining their formal consent to take part in the study. Each participant of the study answered a questionnaire concerning the socioenvironmental and epidemiological characteristics. After the interview, a 10 mL blood sample was requested.

The serum samples were tested for HBV markers using commercially available enzyme immunoassays (DiaSorin, S.p.A., Saluggia, Italy), following the procedures recommended by the manufacturer. All the serum samples were tested for quantitative hepatitis B surface antibody (anti-HBs), total antibody to hepatitis B core antigen (anti-HBc), and HBsAg. Anti-HBs was defined as positive if the result was higher than 10 IU/mL.16 Those reactive to total anti-HBc were tested for total antibody to hepatitis D (anti-HD), and all the samples that were reactive for HBsAg were tested for hepatitis B “e” antigen (HBeAg) and antibody to HBeAg (anti-HBe).

In the HBsAg-positive samples, the HBV DNA levels were quantified by the COBAS AmpliPrep-COBAS TaqMan Hepatitis B virus (HBV) test (CAP/CTM 48; Roche Molecular Systems, Inc., Branchburg, NJ), a fully automated platform for HBV DNA quantification in plasma with a capacity of a lower limit of detection of 12 IU/mL and an upper limit of quantification of 1.10 × 108 IU/mL (conversion factor = 5.82 copies/IU).

The PreS and S regions of the surface gene were amplified by polymerase chain reaction, using the primers 783 [antisense]
brush, use of illegal drugs, and personal history of clinical hep-
surgical interventions, having a tattoo, habit of sharing a tooth-
HBV serological status, vaccination against HBV, previous
handling; the explanatory variables included gender, age group,
P study design. Differences of
intervals (95% CI) were estimated, taking into account the
cant. The Epi Info version 3.3.2 software 20 was used for data
joining method. The phylogenetic groups were evaluated by
ysis with the NCBI tool (http://www.ncbi.nlm.nih.gov/projects/
genotyping/formpage.cgi). The phylogenetic relationship of
Di– X-Terminator (Applied Biosystems), according
to the manufacturer’s instructions. The sequences were per-
formed in an automatic Sequencer ABI PRISM 3130 XL
etic analyzer (Applied Biosystems).

The sequences were edited and aligned using a BioEdit
Sequence Alignment Editor, version 7.0.9.0.18 The HBV
genotype was determined by nucleotide sequencing and anal-
ysis with the NCBI tool (http://www.ncbi.nlm.nih.gov/projects/
genotyping/formpage.cgi). The phylogenetic relationship of
the S gene fragment sequences was determined using Molec-
ular Evolutionary Genetics Analysis (MEGA), version 5.19 The
Tamura–Nei algorithm was used, employing the neighbor-
joining method. The phylogenetic groups were evaluated by
the bootstrap test (1,000 bootstrap replicates).

The prevalence rates of the two outcomes, anti-HBe-reactive
and HBsAg-reactive and their corresponding 95% confidence
intervals (95% CI) were estimated, taking into account the
study design. Differences of \( P \leq 0.05 \) were considered signifi-
cant. The Epi Info version 3.3.2 software20 was used for data
handling; the explanatory variables included gender, age group,
HBV serological status, vaccination against HBV, previous
surgical interventions, having a tattoo, habit of sharing a tooth-
brush, use of illegal drugs, and personal history of clinical hep-
attis. The variable categories were analyzed using logistic
regression, calculating the odds ratio (OR), and 95% CI for
the two outcome variables. Multiple logistic regression models,
including study variables with \( P \leq 0.10 \), were designed to con-
trol for confounding variables using Stata/IC 10.0 software.21

This study was reviewed and approved by the Research
Ethics Committee of Fundação de Medicina Tropical Dr Heitor
Vieira Dourado (FMT-HVD), Manaus, Amazonas, Brazil
(no.: 1775/2006/FMT).

RESULTS

Altogether, we evaluated 225 individuals in the three villages
visited; 115 individuals (51.1%) in the village of Madeirinho;
59 (26.2%) in the village of Praia do Buraco; and 51 (22.7%)
in the village of Samãµma (Figure 1). Of the total, 121 (53.8%)
were male; ages ranged from 1 to 78 years, with a mean age of
21.3 years and a median of 15 years. There were no differ-
ences between the villages in terms of gender and age.

The overall prevalence of HBV infection and HBsAg car-
riage was 79.1% (95% CI = 78.50–79.70) and 10.2%, 95% CI =
8.96–11.44 (23 of 225), respectively. Among the HBsAg car-
riage, the prevalence of HBeAg was 47.8% (11 of 23), and the
mean age of these individuals was 10.8 years (1–36 years).

Chronic HBV infection, positivity for HBsAg, was associ-
ated with the Madeirinho community (\( P = 0.02 \)); the presence
of an HBeAg-reactive individual in the family (\( P < 0.001 \)), and
history of vaccination against hepatitis B (\( P = 0.02 \); Table 1).

Multiple logistic regression analysis showed that HBV
past infection was independently associated with increased
age, the study community Madeirinho, and the presence of
an HBeAg-reactive individual in the family (Table 2). For
HBsAg carriers, the multiple logistic regression analysis showed
an independent association with only the presence of an
HBeAg-reactive individual in the family (Table 1).

All of the HBsAg-reactive samples were positive for
HBV DNA; it was possible to determine the genotype in
65.2% (15 of 23) of the samples, that is, from those individuals
who presented a viral load > 400 IU/mL (Table 3). The
nucleotide sequences ≥ 200 basepair (bp) were deposited
in the GenBank with the following access nos.: 17 LBra
(JQ246014); 26 LBra (JQ246015); 36 LBra (JQ246016); 38
LBra (JQ246017); 44 LBra (JQ246018); 45 LBra (JQ246019),
46 LBra (JQ246020), 68 LBra (JQ246021), 70 LBra (JQ246022),
74 LBra (JQ246023), 75 LBra (JQ246024), 78 LBra
(JQ246025), 124 LBra (JQ246026), 138 LBra (JQ246027). Of
the 15 samples sequenced, 14 were genotype F and 1 was
genotype D (Table 3). Those classified as genotype F showed
higher genomic similarities (Figure 2).

Of the HBsAg carriers, 82.6% (19 of 23) were detected in
households with two to five carriers per family. In three of the
families, the mothers were HBsAg carriers, the two had an HBV
load below the limit of detection (< 12 IU/mL), whereas the
thirds was over 2,000 IU/mL. In the other families, the car-
riers were children or adolescents up to the age of 16 years.
In the HBeAg-reactive individuals, the HBV viral load was
> 10,000 IU/mL (Table 3).

The overall prevalence of the IgG antibodies to HDV
ranged from 3.5% in Madeirinho to 7.8% in Samãµma and
6.8% in Praia do Buraco. The presence of anti-HD was 7.3% (95% CI = 5.89–8.71) among the anti-HBe carriers and 21.7% (95% CI = 19.85–23.55) among individuals HBsAg positive.
The HBV viral load among all the HBsAg/anti-HD positive individuals, except one, was below the detection limit of the test (<12 IU/mL).

DISCUSSION

When the occurrence of HBV was first described in the Brazilian Amazon region, at the end of the 1960s, its epidemiology was associated with rural communities.\(^{15,22-26}\) In this study, it was possible to investigate the epidemiological aspects of HBV infection in the general populations of three rural communities on the Purus river basin, in the municipality of Itapiru, western Amazon, Brazil, using molecular epidemiology as a tool to interpret the distribution and transmission dynamics of HBV infection.

The overall prevalence rate of previous HBV infection 79.1%, and 10.2% of HBsAg carriage can still be classified as a pattern of high endemicity,\(^{21}\) even 20 years after the introduction of the hepatitis B vaccine in the region. This differs from the declines in prevalence described worldwide,\(^{20,27}\) as well as in the rural communities of Southeast Asia,\(^{33}\) and even in the Amazon region.\(^{34}\)

Analysis of the prevalence of the marker of previous infection enables us to infer aspects of HBV distribution in the population studied. Although in the univariate analysis, history of hepatitis B vaccination shows a protective effect, the multiple logistic regression model shows that factors such as age, location, and the presence of an HBeAg-reactive carrier in the family have influenced these results, revealing that the virus still circulates with significant intensity in all age ranges, regardless of the protective effect of the vaccine. There were, however, important differences between the villages; the prevalence of total anti-HBe in the Madeirinho community was around eight times higher compared with Praia do Buraco.

The HBV infection rates observed in these three villages are among the highest ever reported nationally or worldwide, including in the Amazon and in the countries of Southeast Asia, where the reported epidemiological profiles also associate HBV with rural areas.\(^{25,26,35-38}\)

The presence of HBsAg, the most commonly used marker to determine present infection, is an important tool for evaluating the mechanisms of transmission dynamics, its principal actors, and for identifying the population at potential risk of chronic liver disease.\(^{39}\)
**Table 2**

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>N(%) (95%CI)</th>
<th>Crude OR (95%CI)</th>
<th>P value</th>
<th>AOR (95%CI)*</th>
<th>P value</th>
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<td>Total sample</td>
<td>225</td>
<td>178 (79.1) (78.50–79.70)</td>
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<td>4.27 (1.07–17.00)</td>
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<td>10.97 (1.71–69.20)</td>
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<td>22 (61.1) (59.06–63.14)</td>
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<td>8.73 (1.65–46.23)</td>
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<td>10–14</td>
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<td>29 (80.6) (79.16–82.94)</td>
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<td>26.38 (4.18–156.81)</td>
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<td>18 (81.8) (80.02–83.58)</td>
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<td>24.86 (2.98–206.90)</td>
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<td>P. do Buraco</td>
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<td>100 (87.0) (86.54–88.66)</td>
<td>3.96 (1.85–8.45)</td>
<td>&lt;0.001</td>
<td>8.36 (2.58–27.06)</td>
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<tr>
<td>Gender</td>
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<td>M</td>
<td>121</td>
<td>102 (84.3) (83.59–85.01)</td>
<td>1.97 (1.02–3.80)</td>
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<td>2.52 (1.03–6.13)</td>
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<td>F</td>
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<td>Blood transfusion</td>
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<td>1.11 (0.30–3.98)</td>
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<td>45 (75.0) (73.74–76.26)</td>
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<tr>
<td>Yes</td>
<td>165</td>
<td>134 (81.2) (80.54–81.82)</td>
<td>1.38 (0.68–2.79)</td>
<td>0.36</td>
<td>0.83 (0.30–2.28)</td>
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<td>Hepatitis B vaccine</td>
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<tr>
<td>No</td>
<td>66</td>
<td>62 (93.9) (93.30–94.50)</td>
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<tr>
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<td>159</td>
<td>116 (73.0) (72.19–73.81)</td>
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<td>0.27 (0.06–1.19)</td>
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<td>21 (91.3) (89.99–92.51)</td>
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<td>57 (93.4) (93.23–93.57)</td>
<td>5.06 (1.73–14.79)</td>
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<td>9.62 (2.13–43.74)</td>
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</tr>
</tbody>
</table>

Anti-HBC = antibody against hepatitis B core antigen; N = number of subjects; N+ = number of positive subjects; F = female; M = male; 95% CI = 95% confidence interval; AOR* = adjusted for the following variables: age = village, sex, past of clinical Malaria, vaccination against HBV, and HBeAg-reactive in the Family; P value = statistical significance.

**Table 3**

<table>
<thead>
<tr>
<th>ID(IF)</th>
<th>Village</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Degree of Parenthood</th>
<th>History of vaccine</th>
<th>Presence of HBeAg</th>
<th>HBV DNA (IU/mL)</th>
<th>HBV genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 L.Bra (3M) Madeirinho 11 F Daughter Y Pos 1.8 × 10^7 F</td>
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<tr>
<td>25 L.Bra (5M) Madeirinho 14 F Daughter Y Neg 402 F</td>
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<tr>
<td>26 L.Bra (5M) Madeirinho 11 M Son Y Pos 6.12 × 10^4 F</td>
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<td>29 L.Bra (5M) Madeirinho 13 M Daughter Y Neg &lt;12.0 ND</td>
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<td>36 L.Bra (6M) Madeirinho 3 M Son Y Pos &gt;1.10 × 10^8 F</td>
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<tr>
<td>45 L.Bra (7M) Madeirinho 4 M Son Y Pos &gt;1.10 × 10^8 F</td>
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<td>68 L.Bra (9M) Madeirinho 8 M Son Y Pos 4.2 × 10^4 F</td>
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<tr>
<td>70 L.Bra (9M) Madeirinho 5 F Daughter Y Pos &gt;1.10 × 10^8 F</td>
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<td>71 L.Bra (10M) Madeirinho 36 F Mother N Neg &lt;12.0 ND</td>
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<td>74 L.Bra (10M) Madeirinho 5 M Son Y Pos &gt;1.10 × 10^8 F</td>
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<td>78 L.Bra (10M) Madeirinho 14 F Daughter Y Pos &gt;1.10 × 10^8 F</td>
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<td>124 L.Bra (2S) Samauma 29 F Mother Y Neg 5.820 D</td>
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<td>138 L.Bra (6S) Samauma 13 F Daughter Y Neg 5.420 F</td>
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<td>141 L.Bra (6S) Samauma 11 F Daughter Y Neg &lt;12.0 ND</td>
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<tr>
<td>145 L.Bra (6S) Samauma 6 F Daughter Y Pos &lt;12.0 ND</td>
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<td>203 L.Bra (7B) P. do Buraco 39 M Son Y Neg &lt;12.0 ND</td>
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<tr>
<td>209 L.Bra (10B) P. do Buraco 44 F Mother N Neg &lt;12.0 ND</td>
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ID(IF) = sample (family number); M = male; F = female; plasma DNA-HBV viral load = IU/mL; * > upper (above range) limit of the assay; ** lower (below range) limit of the assay; Neg = negative; Pos = positive; ND = not determined.
This study found a population of extremely young individuals with chronic HBV infection, 43.5% were < 10 years of age. Although univariate analysis reveals differences in the distribution of HBeAg between the locations studied and the history of hepatitis B vaccination, these results appear to have been influenced by the presence of a potential transmitter within the family, identified as probably the most significant factor associated with HBV transmission in the region.

Our findings suggest that HBV is circulating with significant intensity in the region, although the problem appears to be greater in certain locations. The prevalence of total anti-HBc was high in all three communities, but with significant differences between villages and age of the individuals, whereas the prevalence of HBsAg was independently associated with the presence of a household of an HBeAg-reactive carrier. This indicates that, in this region, the condition of being a chronic HBV carrier, despite being associated with early age at which the infection occurs, is probably also associated with the frequency and intensity of contacts with HBV.

The characteristics of the potential transmitters (HBeAg-reactive and with a high HBV viral load) suggests that transmission occurs mainly in early ages and within a household as they cluster in families. Vertical transmission may be also possible, because carriers below 4 years of age were identified, however, most of the mothers who are carriers had a low viral load. Nevertheless, vertical transmission has been described from mothers who are carriers of isolated anti-HBc.

It was not possible to identify the factors that facilitate the increased risk for horizontal transmission within families, particularly between individuals from 5 to 14 years of age, in communities where there were no reports of the practice of tattooing, piercing, use of injectable or inhalable drugs, risky sexual behaviors, or practices that facilitate contact with bodily secretions. This heightened transmission is probably associated, first and foremost, with failures in the prevention and control program and, second, with the existence of pockets of high numbers of individuals who are HBV carriers.

The findings of predominantly genotype F, regardless of the community studied, and the genomic similarity between the isolates analyzed suggest that HBV was introduced to these communities relatively recently, from a common source. Genotype F has frequently been described in the Amazon, particularly among native populations. Genotype D was found in an isolate from an individual who was not native to the community studied and is similar to genotypes found in the Mediterranean and East Africa. It was probably introduced to the region by the Lebanese peddlers during the “Rubber Cycle.”

The molecular data confirmed the household nature of HBV transmission in this population, as described previously in the Amazon. However, among children 1 to 14 years of age, in which the majority of chronic carriers and potential transmitters were concentrated, it was not possible to identify the transmission mechanisms. Studies carried out in the Amazon region suggest that HBV transmission is associated with living with an HBV carrier and with sharing items of personal use.

We observed a high prevalence rate of total anti-HDV in these three areas. Notably, similar rates were reported in the Amazon, suggesting that HDV has not yet disappeared from HBV hyperendemic areas. This may have an important health burden because HDV is an established cause of severe liver injuries. Nonetheless, HDV epidemiology may differ in other areas of the Brazilian western Amazon region. The low HBV viral load among those HBsAg positive individuals is probably caused by the HDV coinfection that may spontaneously suppress HBV.

This study is a continuation of two previous studies to define the most important aspects of HBV infection epidemiology in this region described as highly endemic. A pattern is outlined in which pockets of chronic HBV carriers are identified where potential reservoirs are concentrated. The importance of vertical transmission in the region has also been determined, and is probably one of the mechanisms responsible for maintaining HBV circulation in populations at high risk of developing liver cirrhosis and hepatocellular carcinoma caused by the burden of HDV infection.

The continued expansion of migrations of individuals from HBV endemic areas has a significant impact on the epidemiology and increased prevalence of chronic hepatitis B in areas previously considered non-endemic.
complex changes, bringing opportunities and risks to the health of populations. Universal child immunization is the most effective way of reducing the global prevalence of HBV infection. The globalized community should assist resource-limited areas where programs to control HBV have been unsuccessful to eradicate pockets of ongoing transmission of HBV compromising its global control.

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REFERENCES


