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Major Article

Hepatitis D virus infection in the Western Brazilian Amazon - far from a vanishing disease

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ABSTRACT

Introduction: A decline in hepatitis D virus (HDV) occurrence was described in Europe and Asia. We estimated HDV prevalence in the Brazilian Amazon following hepatitis B vaccination. **Methods:** This is a cross-sectional survey of HDV measured by total antibodies to HDV (anti-HD T). **Results:** HDV prevalence was 41.9% whiting HBsAg carries and was associated with age (PR = 1.96; 95% CI 1.12-3.42; p = 0.01), hepatitis B virus (HBV) infection (PR = 4.38; 95% CI 3.12-6.13; p < 0.001), and clinical hepatitis (PR =1.44; 95% CI 1.03-2.00; p = 0.03). Risk factors were related to HDV biology, clinical or demographic aspects such as underlying HBV infection, clinical hepatitis and age. **Conclusions:** Our study demonstrated that HDV infection continues to be an important health issue in the Brazilian Amazon and that the implementation of the HBV vaccination in rural Lábrea had little or no impact on the spread of HDV. This shows that HDV has not yet disappeared from HBV hyperendemic areas and reminding that it is far from being a vanishing disease in the Amazon basin.

Keywords: Hepatitis D virus. Viral diseases. Epidemiology.

INTRODUCTION

Hepatitis D virus (HDV) is a singular human pathogen. It is a sub-satellite RNA virus and presents a fascinating biological cycle dependent on the hepatitis B virus (HBV) which is still poorly understood. Approximately 5% of the 300 million HBV carriers worldwide are estimated to be positive for HDV[1]. Clinically, HDV infection exacerbates the course of HBV-associated liver disease and it is very common for fatal acute cases to develop with early progression to end-stage chronic liver disease with little choice of effective therapies[2].

High prevalence rates are reported within regions of important HBV surface antigen (HBsAg) carriage, such as the Amazon region[3,4], Sub-Saharan Africa[5,6], and Eastern Europe[7]. More recently, HDV has been described in regions of high endemicity of HBV without previous screening for HDV serological markers, such as Greenland[8] and Siberia[9].

In the western Brazilian Amazon region, family outbreaks of fulminant hepatitis, called Lábrea black fever, used to be very common in small rural villages[10,11]. Recently this disease was reported in the Amazon region of Ecuador[12].

A decline in HDV prevalence and incidence are described in countries of Europe and Eastern Asia due to improved HBV control with the HBV vaccine in National Immunization Programs[13,14]. Nevertheless, immigration from eastern countries has threatened the HBV and HDV control policies in Europe[15].

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This study aimed to estimate the prevalence of HDV serological markers and risk of infection in the rural zone of the Lábrea County, located in the south western Brazilian Amazon, following 19 years of hepatitis B vaccination in the region.

METHODS

Study design and study area

This was a descriptive cross-sectional survey of HDV distribution and risk of infection in the rural population of Lábrea. Total antibodies to HDV (anti-HD IgG) among subjects showing serological evidence of past or chronic HBV infection 19 years after the HBV vaccination program began were measured.

Lábrea is located in the south western Brazilian Amazon (**Figure 1**) and has an estimated rural population of 10,000 inhabitants. Historically, the region was first colonized by people collecting forest products. In the nineteenth century it was occupied by the resettlement of a large number of immigrants from northeast Brazil during the rubber boom.

Currently, the villages are small and are formed from families staying after the end of the rubber boom. The economy is still based on the exploitation of natural resources and family agriculture. Socio-economic conditions are very poor.

Study population and field work

This investigation includes only individuals at risk of HDV infection. The target population was those, irrespective or their ages, showing serological markers of a previous or chronic HBV infection. The participants were selected as a sub-population of the core population of a cross-sectional survey of HBV prevalence.

Villages and households along the banks of the Purus River were randomly chosen to represent the entire geographical area

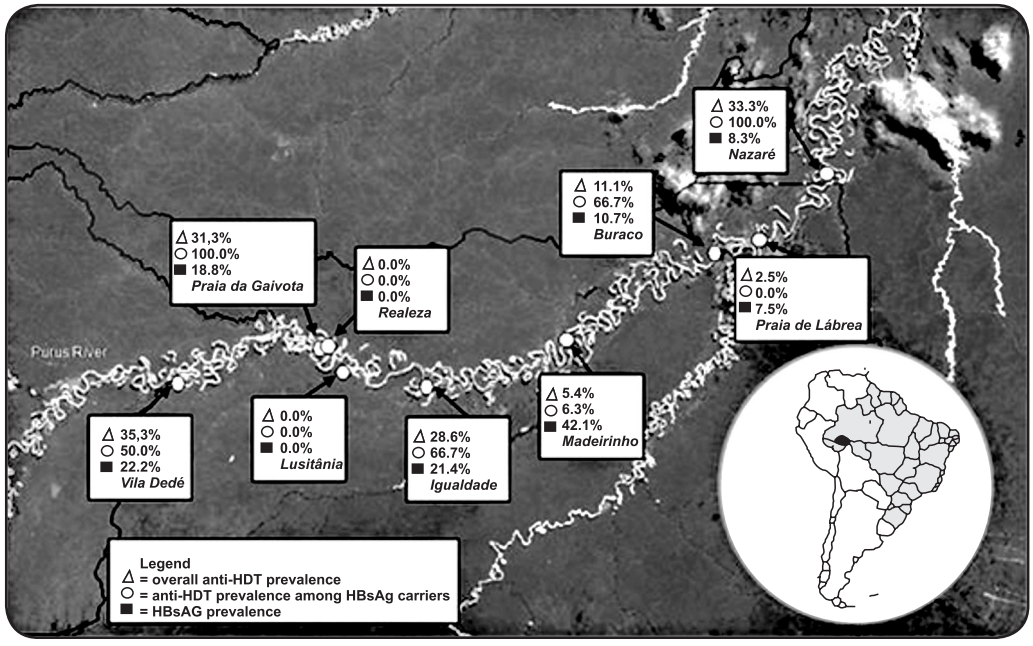

FIGURE 1 - Study area and hepatitis D virus prevalence in some visited villages.

of the province. The villages were visited from March 2005 to April 2006 during three twenty day-expeditions and a few one-day trips to the villages near the City of Lábrea.

Sample collection

All the participants gave an informal consent and filled individual questionnaires, 10mL blood samples from all the participants were taken during the initial visit. Some family members were included at a later stage because of their absence at the initial visit. None of the selected family refused to participate of the study. Data and blood sample collection ended once all the selected villages had been visited.

Number of studied subjects

Sample size calculations were not performed. The number of study subjects included was dependent on the sample size of the core study and was also related to the overall prevalence of HBV infection. Since HBV was measured with probabilistic parameters and all of the individuals included in this study were selected as a sub-population of those at risk of HDV infection, the studied sample was representative of those at risk of HDV infection and less likely to be influenced by bias due to the size of the sample analyzed.

Serological tests

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-hepatitis B surface (anti-HBs) and total anti-hepatitis B core (anti-HBc). Those reactive to total anti-HBc and negative for anti-HBs were tested for hepatitis B virus surface antigen (HBsAg). All samples reactive for total anti-HBc were also tested for anti-HD IgG using a commercial kit (DiaSorin, S.p.A., Saluggia, Italy). The test procedures followed manufacturer's recommendations and were performed automatically at the laboratory facilities of the virology unit of FMT-HVD.

Statistical analysis

The study population was stratified according to HBV serological status as follow. Group 1 total anti-HBc and anti-HBs reactive, group 2 isolated total anti-HBc reactive and group 3 HBsAg reactive. In order to fit logistic regression models and to allow testing for statistical interaction, age was classified as a binary variable 0 for those up to 14 years old, and 1 for those 15 years or older.

Prevalence rates along with corresponding 95% confidence intervals (95% CI) were estimated taking into accounting the study design.

The Epi Info™ version 3.3.2 software[16] was used for data handling; explanatory variables included gender, age group, type of HBV infection, vaccination against hepatitis B, past surgical interventions, having a tattoo, habit of sharing a toothbrush, use of illicit drugs, and personal antecedents of clinical hepatitis or jaundice. The variable categories were analyzed using Poisson regression with robust variance estimates, calculating Prevalence Ratio (PR) and 95% CI. A final multiple Poisson regression model including study variables with a p-value equal to or less than 0.10 was designed to control for confounding factors using Stata/IC 10.0 software[17].

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 787 individuals from 298 families, comprising 52.1% of the population of the core study. The median age of the participants was 29 years (1-87 years).

TABLE 1 - Hepatitis D (anti-HD IgG reactive) prevalence and associated variables, rural Lábrea municipality, western, Brazilian Amazon region, 2006.

Variable	N	N^+	%	95%CI	PR*	95%CI	p-value
Type of HBV infection							
I	594	55	9.3	8.5–10.1	1		
II	90	11	12.2	10.3–14.3	1.32	0.71–2.42	
III	93	39	41.9	40.4–43.5	4.53	3.19–6.41	<0.001
Age group (years)							
<14	180	14	7.7	6.3–9.1	1		
≥15	597	91	15.2	14.5–15.9	1.96	1.14–3.36	0.01
Gender							
female	385	57	14.8	13.9–15.7	1		
male	392	48	12.2	11.3–13.1	0.83	0.58–1.18	0.29
Past hepatitis							
no	582	64	11.0	10.2–11.7	1		
yes	181	39	21.5	20.2–22.8	1.96	1.36–2.81	<0.001
Past surgery							
no	658	84	12.8	12.9–13.5	1		
yes	114	21	18.4	16.7-20.1	1.44	0.93–2.23	0.10
Past HBV vaccine							
no	246	34	13.8	12.6–14.9	1		
yes	507	69	13.6	12.8–14.4	0.98	0.67–1.44	0.94
Tattoo							
no	743	101	13.6	12.9–14.3	1		
yes	26	3	11.5	4.9–18.2	0.84	0.28–2.60	0.76
Sharing toothbrush							
no	618	81	13.1	12.4–13.8	1		
yes	152	23	15.1	13.6–16.6	1.15	0.75–1.77	0.51
Use of illegal drugs							
no	643	89	13.8	13.1–14.5	1		
yes	5	1	20.0	12.2–27.8	1.44	0.24–8.44	0.68
Total sample	**787**	**105**	**13.5**	**10.1–16.9**			

Anti-HD IgG: Total antibodies against Delta antigen. **Type of hepatitis B virus infection**: **I** - resolved infection; **II** - total antibodies against hepatitis B virus core antigen alone; **III** - hepatitis B virus surface antigen carriers. **N**: number of subjects; **N^+**: number of positive subjects; **95%CI**: 95% confidence interval; **PR***: crude prevalence ratio; **p-value**: statistical significance.

TABLE 2 - Hepatitis D and associated variables, multiple logistic regression analysis including all variables - rural Lábrea municipality, western, Brazilian Amazon region, 2006.

Variable	PR*	95%CI	p-value	PR**	95%CI	p-value
Type of HBV infection						
I	1			1		
II	1.32	0.71–2.42	<0.001	1.21	0.64–2.31	
III	4.53	3.19–6.41		4.38	3.12–6.13	<0.001
Age group (years)						
≤14	1			1		
≥15	1.96	1.14–3.36	0.01	1.96	1.12–3.42	0.01
Past hepatitis						
no	1			1		
yes	1.96	1.36–2.81	<0.001	1.44	1.03–2.00	0.03
Past surgery						
no	1			1		
yes	1.44	0.93–2.23	0.10	1.17	0.80–1.72	0.39

Type of hepatitis B virus infection: I - resolved infection; **II** - total antibodies against hepatitis B virus core antigen alone; **III** - hepatitis B virus surface antigen carriers; **95%CI:** 95% confidence interval; **PR***: crude prevalence ratio; **PR**:** adjusted prevalence ratio; **P-value**: statistical significance.

The overall HDV prevalence (anti-HD IgG reactive) was 13.5% (95% CI 10.1-16.9).

Overall HDV infection varied significantly between villages from as low as 0% at Realeza (07°35'52.8"S/66°13'29.2"W) to as high as 35.3% at the village of Vila Dede (07°42'33.0"S/66°40'25.2"W).

We detected a heterogeneous pattern of HDV distribution within study villages among HBV carriers. A low HDV circulation profile of less than 10% and a high profile around 100%, for instance, was found at the villages of Vila Madeirinho (07°34'19.1"S/65°26'31.4"W) and Praia de Lábrea (07°15'17.2"S/64°49'53.3"W), and Seringal da Igualdade (07°43'11.1"S/65°53'11.2"W) and Vila Dede (07°42'33.0"S/66°40'25.2"W), respectively (**Figure 1**).

Hepatitis D virus was positively associated with age (chi-square for linear trend = 5.49, p = 0.02), varying from 10.8% (95% CI 7.8--13.8) among those up to 4 years, 7% (95% CI 5.4--8.6) in the group aged 5 to 14 years, 14.3% (95% CI 12.1--16.5) among those 15 to 19 years, 15.7% (95% CI 14.7--16.7) between 20 and 39 years, and 14.9% (95% CI 13.7--16.1) among those 40 years and older.

Hepatitis D virus age distribution among HBsAg carriers varied from 25% (95% CI 16.5--33.5) among those up to 4 years, 8.3% (95% CI 4.5--12.1) in the group aged 5 to 14 years, 40% (95% CI 33.2--46.8) among those 15 to 19 years, 61.5% (95% CI 59.6--63.4) between 20 and 39 years and, 47.6% (95% CI 44.5--50.7) among those 40 years old and older.

In the Poisson regression analysis, HDV infection was significantly associated with age (15 years or older) (PR = 1.96, 95%CI 1.14-3.36, p=0.01), with the type of HBV infection (PR = 4.53, 95% CI 3.19-6.41, p<0.001) among HBsAg carriers, and with a history of previous clinical hepatitis (PR = 1.96, 95%CI 1.36-2.81, p<0.001). The prevalence of HDV was not associated with gender, history of surgery, tattoo and use of illicit drugs, sharing a toothbrush or with previous hepatitis B vaccination (**Table 1**).

After fitting a multiple Poisson regression model to data including age, type of HBV infection, history of past hepatitis and past surgical interventions, HDV infection remained independently associated with the type of HBV infection, past history of hepatitis and older ages (**Table 2**).

DISCUSSION

The study design allowed us to select a representative sample of only those at risk of HDV infection since subjects had previously been screened for serological markers of HBV. This allowed us to estimate HDV impact and to define

epidemiological aspects in an area of high prevalence of both viruses with community based results. It was found that HDV was markedly present in the study population.

We found a very high (13.5%) overall prevalence for total anti-HD. Previous studies with community based parameters reported lower rates, such as 2.9% in the province of Acre in the western Brazilian Amazon[18] and 6.1% in Mongolia among school children[19]. However, this is not a straightforward comparison as the investigations diverged too far regarding study populations, although an important presence of HDV infection in the study areas can be observed. However, HDV epidemiology may differ throughout other areas of the Brazilian western Amazon region[18].

The low (9.3%) prevalence of HDV infection among those with serological profiles of resolved HBV infection may have revealed a small number of cases of simultaneous infections. As HDV has the ability to suppress HBV, the 12.2% infection rate among isolated total anti-HBc subjects may have represented some cases of HDV infection with occult hepatitis B infection.

HBV carriers are the target populations of the majority of studies published, mainly because of the role of high HBsAg prevalence in the biological interactions between HDV and HBV, the dependence of HDV on HBV maintains both presence in the community and burden of clinical outcomes[20,21,22].

We found a prevalence of HDV infection among chronic HBV carriers of more than 40%, and over 60% among individuals aged 20 to 39 years. This is undoubtedly one of the highest ever reported in the era of hepatitis B vaccination. However, it is not easy to establish inferences because studies have tended to evaluate specific cohorts, such as 6.1% among children in Mongolia[19], 15.6% in pregnant women in Gabon[5] or 12% in central Turkey in patients with liver disorders[23]. Only in the Amazon region, other studies have observed similar rates[18,24,25].

Hepatitis D virus has been systematically measured in patients with HBV-related liver disease. It is true that these subjects are at risk of severe outcomes. In this study we found an increased risk for past clinical hepatitis among HBV carriers. However, we also observed that HDV infection occurs in different scenarios with significant intensity. It is likely that this may have some influence on the occurrence of occult hepatitis B in this region. Hepatitis D virus has also been reported elsewhere in large proportions of individuals with mild or no liver damage[26]. Hence, this viral interaction must be evaluated very broadly to meet all possible information for HDV control.

The heterogeneous geographical distribution within villages was probably one of the most interesting findings in this study. It is intriguing how some villages with an increased prevalence of HBV carriage had little to no HDV circulation. HDV mainly spreads through super-infection of HBsAg chronic carriers than simultaneously. Our study is an example of how the HDV-HBV linkage in an area may connect circumstantially among different age cohorts, therefore with different intensities and transmission routes.

Hepatitis D virus was found to be present in very young children but perinatal transmission did not seem to be of a great importance. Hepatitis D virus was only detected in one HBsAg-positive subject up to 4 years, who also had an HBsAg-positive mother. Vertical transmission of HDV is known to be quite an inefficient route. In addition, in the Brazilian Amazon region HBsAg-positive women frequently showed a low HBV viral load and are also antibody to hepatitis B e antigen (anti-HBe) positive in the majority of cases[27].

Hepatitis B virus carriers 15 years old or older presented a risk of HDV infection almost double than those of a younger age. Nevertheless it seemed that there was no cohort effect of HDV infection in the study population and overall prevalence of HDV infection among HBV carriers were quite similar beyond the age of 15 years.

The highest prevalence of HDV infection was described among intravenous drug users before the implementation of the HBV vaccine in Europe[28], suggesting HDV is directly transmitted through blood to blood exposure. In our study, the risk factors were related to the biology of the virus and clinical or demographic aspects, such as underlying HBV infection, history of past clinical hepatitis and age. We did not find any associations between HDV prevalence and history of previous surgeries, tattoos or sharing of personal tools such as a tooth brush, all of which are factors that could have exposed individuals to direct blood contact.

Alternatively, the increased risk to older ages, especially between 20 to 39 years, may indicate the importance of sexual transmission. As HBsAg seemed to cluster within villages and families, intra-familiar dissemination may play an important role once HDV is introduced into the community.

In conclusion, our study demonstrated that HDV infection continues to be an important public health issue in the Brazilian Amazon region, and that the implementation of the HBV vaccination program in rural Lábrea had little or no impact on the spread of HDV, probably due to a lack of efficient HBV control policies. Our findings have global importance in showing that HDV has not yet disappeared from HBV hyperendemic areas. Remarkably it is far from being a vanishing disease in the Amazon basin.

Surveillance of acute and chronic cases, as well as molecular studies is needed to provide a broad understanding of HBV-HDV viral interactions in the region.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABSTRACT IN PORTUGUESE

Infecção pelo vírus da hepatite D na Amazônia brasileira - longe de uma doença em declínio

Introdução: É descrito declínio na ocorrência do vírus da hepatite D (VHD) na Europa e Ásia. Estimamos a prevalência de infecção pelo VHD na Amazônia Ocidental, após a introdução da vacinação contra hepatite B. **Métodos:** Este é um estudo de corte transversal da prevalência do VHD medido pela ocorrência de anticorpos totais (anti-HD T). **Resultados:** A prevalência do VHD encontrada foi 41,9% entre os portadores do HBsAg, e esteve associado à idade (RP = 1,96; IC 95% 1,12-3,42; p = 0,01), infecção pelo HBV (RP = 4,38; IC 95% 3,12-6,13;

p < 0,001) e história clínica de hepatite (RP =1,44; IC 95% 1,03-2,00; p = 0,03). Fatores de risco mostraram-se associados à biologia do HDV, aspectos clínicos e demográficos como infecção prévia pelo VHB e idade. **Conclusões:** O estudo demonstra que a infecção pelo VHD continua sendo um importante problema de saúde pública na região, e que a implantação da vacinação contra o VHB na área rural de Lábrea teve um impacto pouco significativo no controle do VHD, percebe-se que este ainda não desapareceu de áreas hiperendêmicas do VHB, e está longe de poder ser classificado como uma doença em declínio na bacia Amazônica.

Palavras-chaves: Vírus da hepatite D. Doença viral. Epidemiologia.

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