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HIGH PREVALENCE OF HEPATITIS B VIRUS AND HEPATITIS D VIRUS IN THE WESTERN BRAZILIAN AMAZON

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Abstract. Severe cases of hepatitis caused by hepatitis B virus (HBV) or hepatitis D virus (HDV) are often seen in the Brazilian Amazon, but there is a paucity of epidemiologic studies on viral hepatitis in this area. Thus, a cross-sectional study to investigate the prevalence of markers for HBV and HDV was performed. Serum samples were collected after participants completed an epidemiologic questionnaire. Markers for HBV and HDV were analyzed with an enzyme-linked immunosorbent assay. The HBV genotype was determined by sequencing of the gene for hepatitis B surface antigen (HBsAg). Of 2,656 samples, 89 (3.3%) were positive for HBsAg and 1,628 (61.5%) were positive for IgG antibody to hepatitis B core antigen. Markers for HDV were found in 47 cases (1.7%). Antibodies to HDV were associated with Amerindian ethnic origin, a lower educational level, a history of acute viral hepatitis, a history of malaria, male sex, a history of tattooing, and older age. The most frequent HBV genotypes were A and F. This study showed a high prevalence of HBV and HDV in the western Brazilian Amazon, as well as the predominance of HBV genotypes A and F.

INTRODUCTION

Hepatitis D virus (HDV) was first described by Rizzetto and others in 1977.[1] This unique human virus is associated with co-infections or super infections of patients infected with hepatitis B virus (HBV). Hepatitis D virus has a worldwide distribution. In Africa, the Middle East and southern Italy, up to 24% of carriers of hepatitis B virus surface antigen (HBsAg) have markers for HDV. Conversely, infection with HDV is uncommon in the United States, where it is almost restricted to drug users and hemophiliacs, with prevalence rates ranging from 1% to 10%.[2]

In Brazil, HDV is restricted to the western Amazon region, where severe cases of acute and chronic liver disease has been associated with HBV/HDV co-infection or super infection.[3] In this region, a unique form of fulminant HBV/HDV hepatitis, known as Labrea fever, has been described, and its resembles another peculiar form of fulminant hepatitis caused by HDV described in the African equatorial forest.[4–6]

In the State of Acre (in the western Amazon region of Brazil), many clinical epidemiologic surveillance reports have described the occurrence of a so-called severe disease on rubber plantations. Reports from clinicians have cited severe hemorrhagic forms of hepatitis on these plantations. Despite geographic difficulties in organizing health services in this region, some cases were investigated and confirmed by serologic examination as hepatitis caused by HDV (data from the epidemiologic surveillance reports of the Health Secretary Office of the State of Acre[3]).

The purpose of this study was to investigate the seroepidemiologic features of HBV and HDV in this region. These features can contribute to a better understanding of the epidemiologic and clinical aspects of these viruses in the Brazilian Amazon region.

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PATIENTS AND METHODS

Study area. The study area in the western part of the State of Acre in Brazil is shown in Figure 1. The seroepidemiologic survey included 12 of the 22 municipalities in the state and 25% of the population of Acre. Local clinical observations indicated a higher prevalence of hepatitis caused by HDV in this population, with expressive cases of chronic hepatitis and cirrhosis due to the association of HDV with HBV, as well as outbreaks of fulminant hepatitis. The municipalities included in the study comprise a geographic area of approximately 650 km^2. The socioeconomic status of its population is low. Many inhabitants are involved in the rubber industry and cultivation of nuts. This region has poor access to almost all municipalities and poor mobility of its population, in addition to expressive cohabitation with local indigenous peoples.

Study population. The reference population consisted of individuals who resided in the State of Acre for six months or longer. From this group, the study population was selected, which consisted of inhabitants of census sectors defined by the Brazilian Institute of Geography and Statistics (IBGE) for the following municipalities: Assis Brasil, Sena Madureira, Manoel Urbano, Santa Rosa, Feijó, Tarauacá, Jordão, Cruzeiro do Sul, Rodrigues Alves, Mâncio Lima, Porto Walter, and Marechal Thaumaturgo. The census sectors consisted of groups of 200–350 dwellings that according to the IBGE represent the population of the corresponding area.[4]

There are 556 urban and rural census sectors in the State of Acre, some with a radius of 100 km. If one considers a mean number of 250 families per census sector and 5 individuals per family, the size of the population is estimated to be 695,000 inhabitants. Since the expected prevalence of HDV is 0.4%, the minimum sample size would be 1,701 individuals.

The areas and houses were selected by drawing lots according to the estimate previously established for each town. To choose a person to be included in the study from each selected dwelling, all respective dwellers were registered and received a number according to the order of citation by the person responsible for the dwelling. Based on this list, only

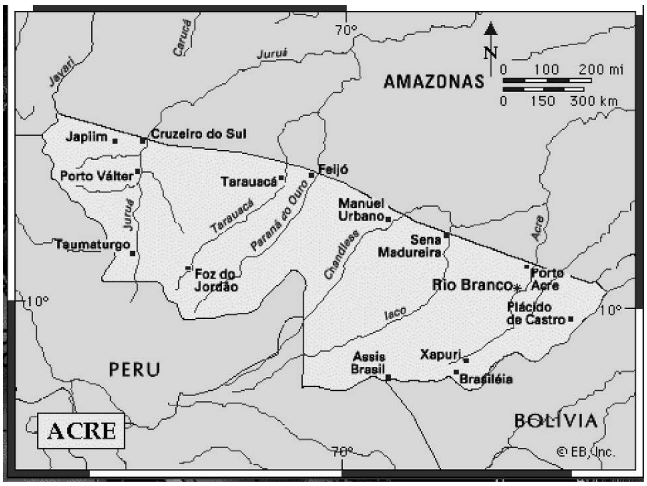

FIGURE 1. State of Acre in the western Amazon region of Brazil.

one of the dwellers was selected by drawing lots without replacement.

Inclusion and exclusion criteria. Inclusion criteria for the selected dweller were residence in the State of Acre for six months or longer and willingness to participate in the study. Participants signed an informed consent form. The study was reviewed and approved by the Research Ethics Committee of the University of Brasilia (according to instruction no. 196, Local Research Ethics Committee/National Research Ethics Committee, Brazilian Ministry of Health). Exclusion criteria were a temporary residence in the State of Acre or residence for less than six months and a refusal to participate in the study. In addition, children without a responsible guardian and individuals with some cognitive deficit without a responsible person present at the time of the interview were also excluded.

Study design. The selected individuals were interviewed by one of the researchers participating in the study, who completed an epidemiologic questionnaire that included demographic (age, sex, place of birth, origin, occupation, indicators of human development, time of residence in forest areas) and clinical/epidemiologic (history of malaria, risk factors associated with transmission by hepatotropic viruses, history of hepatitis) variables. After this step, blood samples were collected.

Blood samples were divided into two aliquots (1–2 mL) and stored at 4–8°C until the time of transportation to the laboratory in Rio Branco, the state capital. The samples were transported in thermos bottles on ice to maintain a temperature less than 10°C to maintain the quality of the samples for serologic and biomolecular analysis. In Rio Branco, the samples were stored at –20°C until transported in a polystyrene box containing dry ice to the Laboratory of Viral Hepatitis Adolfo Lutz Institute in Sao Paulo.

Participants were classified as Amerindians (Indians and mixed race Indians) and non-Amerindians (Blacks and Caucasians). The location of the dwelling (rural or urban) was defined based on IBGE data, with rural dwellings being those located outside the urban perimeter delimited in each locality.

The municipality of Santa Rosa was first chosen of its geographic isolation and its location outside Rio Purus, an area where many cases of hepatitis caused by HBV and HDV, including fulminant hepatitis, have been recorded.

Serologic markers. Serologic markers for HDV were analyzed using commercial kits (Abbott, Abbott Park, IL). All HBV markers were analyzed using commercial kits (Organon Teknika, Boxtel, The Netherlands). The following serologic markers were analyzed: total antibody to hepatitis B virus core antigen (anti HBc), HBsAg, antibody it HBsAg, and antibody to HDV. When an individual was positive for a single marker, the samples were successively confirmed with careful analysis of the optical density:cut-off ratio. Undetermined results (low optical density:cut-off ratio) were excluded from the final analysis.[6]

Detection of HBV DNA by PCR. Samples with HBsAg-positive results were amplified for the S gene by a nested polymerase chain reaction (PCR) as described by Kaneko and others.[7] Primers used were described by Sitnik and others.[8] The material obtained by a second PCR for amplification of the S region (20 μL) was precipitated by the addition of 80 μL of 95% ethanol at –20°C. The samples were then incubated on ice for 15 minutes and centrifuged at 16,060 × *g* for 20 minutes at room temperature. The pellet was washed with 250 μL of 70% ethanol and centrifuged again for 5 minutes at room temperature. The pellet was then resuspended in 20 μL of deionized water.

Sequencing reaction (cycle sequencing). The PCR products were used to for cycle sequencing as decribed by Sanger and others[9] using the second-round primers described by Sitnik and others,[8] fluorescence-labeled ddNTPs and the ABI Prism BigDye Terminator ready reaction kit (Perkin Elmer Applied Biosystems, Foster City, CA). Briefly, 2 μL of sample containing 3–10 ng of DNA was mixed with 2 μL of the HBS2F and HBS2R (S) primers, 4 μL of the sequencing reaction mixture containing labeled ddNTPs, AmpliTaq DNA polymerase, pyrophosphatase, 4 μL of Tris-HCl buffer, pH 9.0, $MgCl_2$, and 8.0 μL of deionized water in a final volume of 20 μL. The samples were then incubated in a thermocycler using a specific program for complementary strand synthesis and incorporation of the labeled ddNTPs.[7]

Sequence analysis. Genotypes were analyzed by comparison of the sequences obtained with other known sequences from different HBV genotypes deposited in GenBank, using the EditSeq and Megalign programs included in the DNAstar package (Lasergene, Inc., Madison, WI).

Statistical analysis. Qualitative or quantitative variables were analyzed using nonparametric tests, the chi-square test, or the Mann-Whitney test as indicated. Quantitative variables were also analyzed with the Student *t*-test or correlation test. Differences were considered significant when the probability (*P*) of a type I error was 0.05 (5%). Since explicative variables and the response variable (total antibody to HDV) are categorized, the chi-square test was initially used to determine the correlation between these parameters. Odds ratios and 95% confidence intervals (CIs) were calculated.

RESULTS

The study was carried out between February 17 and July 20, 2002. A total of 2,754 epidemiologic questionnaires were completed, but 25 individuals refused to provide a blood sample, 62 had an undetermined result upon analysis, and 21

had serum samples that were insufficient for all tests. These individuals were excluded from the analysis.

Of the 2,656 individuals studied, 57.9% were females and 42.1% males. The mean ± SD age of the population was 32 ± 17.9 years (range = 0–92). With respect to race, 2,552 (96.0%) were non-Amerindians (74.4% African Brazilians and 25.6% Caucasians) and 4.0% (n = 104) were Amerindians.

A total of 1,628 (61.2%) samples were positive for antibodies to HBc. The prevalence of antibody to HBc ranged from 45.3% to 89.7% depending on the area studied. The presence of antibodies to HDV was determined in 1,628 individuals positive for IgG antibodies to HBc. Of these, 47 (2.9%) were reactive, but only 19 were concomitantly positive for HBsAg. The overall prevalence of antibodies to HDV in the total population was 1.8%. The prevalence of the IgG antibodies to HDV ranged from 1.02% in Sena Madureira to 8.16% in Manoel Urbano. However, HDV was not detected in the Mâncio Lima.

Hepatitis B virus surface antigen was detected in 89 (3.3%) of 2,656 individuals, which corresponded to 5.4% of the individuals positive for IgG aantibodies to HBc. Genotyping of HBV was conducted in 34 of the HBsAg carriers, Nine individuals, most of them Amerindians, had genotype F and 25 had genotype A (Table 1). Antibody to HBs was detected in 31.2% of the individuals. This was probably the result of the hepatitis B vaccination program initiated in the State of Acre in 1999. Figure 2 summarizes the results of the seroprevalence of HBV and HDV markers.

TABLE 1
Genotyping results obtained by amplification and sequencing of the pre-S region of hepatitis B virus*

Sample number	Subtype	Genotype
CS 27	Adw2	A
CS 35	Adw4	F
CS 44	Adw2	A
CS 53	Adw2	A
CS 152	Adw4	F
CS 373	Adw2	A
CS 428	Adw2	A
CS 705	Adw2	A
CS 713	Adw2	A
CS 719	Adw2	A
CS 726	Adw4	F
SM 184	Adw4	F
SM 308	Adw2	A
F 10	Adw2	A
F 89	Adw2	A
F 93	Adw2	A
F 212	Adw2	A
F 276	Adw2	A
T 66	Adw2	A
T 78	Adw2	A
T 205	Adw2	A
T 218	Adw2	A
T 257	Adw2	A
T 263	Adw2	A
MT 3	Adw4	F
MT 79	Adw4	F
MT 87	Adw4	F
RA 59	Adw4	F
RA 81	Adw2	A
J 34	Adw2	A
J 38	Adw2	A
PW 11	Adw2	A
PW 56	Adw2	A
AB 21	Adw4	F

* CS = Cruzeiro do Sul; SM = Sena Madureira; F = Feijó; T = Tarauacá; MT = Marechal Thaumaturgo; RA = Rodrigues Alves; J = Jordão; PW = Porto Walter; AB = Assis Brasil.

A higher proportion of individuals positive for antibodies to HDV was observed among males (4.3%) compared with females (1.8%) ($P < 0.001$), and the risk of having total antibodies to HDV was 3.1 times higher among men. The mean age of individuals positive for antibody to HDV was 38.6 years, and the mean age of nonreactive individuals was 31.5 years, a difference of 7.1 years ($t = 2.65$, $P < 0.004$). Grouping of individuals by age showed a progressive increase in the proportion of individuals positive for antibodies to HDV with age.

The prevalence of antibodies to HDV was 2.5% (n = 13) in the group that never lived in a rural area and 2.8% (n = 29) in the group that did. The odds ratio (lived/never lived in rural areas) was 0.875 (95% CI = 0.451–0.169). Antibody to HDV was less frequent among non-Amerindians (2.2%, n = 40) than among Amerindians (7.1%, n = 7) ($P < 0.002$). The odds ratio for Amerindians versus non-Amerindians was 3.45 (95% CI = 1.505–7.913). Furthermore, the level of schooling, measured by number of years, showed a significant correlation with the presence of antibodies to HDV: 3.4% (n = 31) of reactive individuals had four or less years of schooling, and 1.5% (n = 15) had five or more years of schooling ($P < 0.008$) (Table 2).

An association was observed between a history of jaundice and positive serologic results for antibodies to HDV ($P < 2.2 \times 10^{-10}$). A history of jaundice was reported by 346 individuals. Of these, 22 (7.0%) were positive for antibodies to HDV, compared with only 35 (1.5%) of 2,310 without a history of jaundice.[9] A correlation was observed between hunting, fishing, or camping and positive serologic results for antibodies to HDV ($P < 0.005$). The clinical and epidemiologic variables associated with HDV seropositivity are shown in Table 3.

DISCUSSION

Infection with HBV and HDV is under control in developed countries, but it is a serious public health problem in developing countries.[10–12] In the specific case of the Amazon region of Brazil, data indicate an increase in hepatitis B and D, and both are considered serious public health problems despite universal vaccination policies in some places.[13,14] The epidemiology of HBV and HDV in Brazil and South America needs to be clarified. Hepatitis B virus is highly endemic in the Amazon Basin, but HDV is restricted to the western Amazon region. Up to now, HDV was not considered endemic in other South American areas.

The results of this study confirm the high prevalence of HBV and HDV in the State of Acre. The municipalities in this state have populations that have increased since the end of the 19th century and the beginning of the 20th century by migration into the region. Hepatitis B and D viruses may have been introduced into the region during the increase in migration involved with the rubber industry, but they remained isolated in some communities until the present time. Also, modernization of transportation in this region may have resulted in the spread of these viruses. Most towns in this region do not have access to regular roads, with most communication

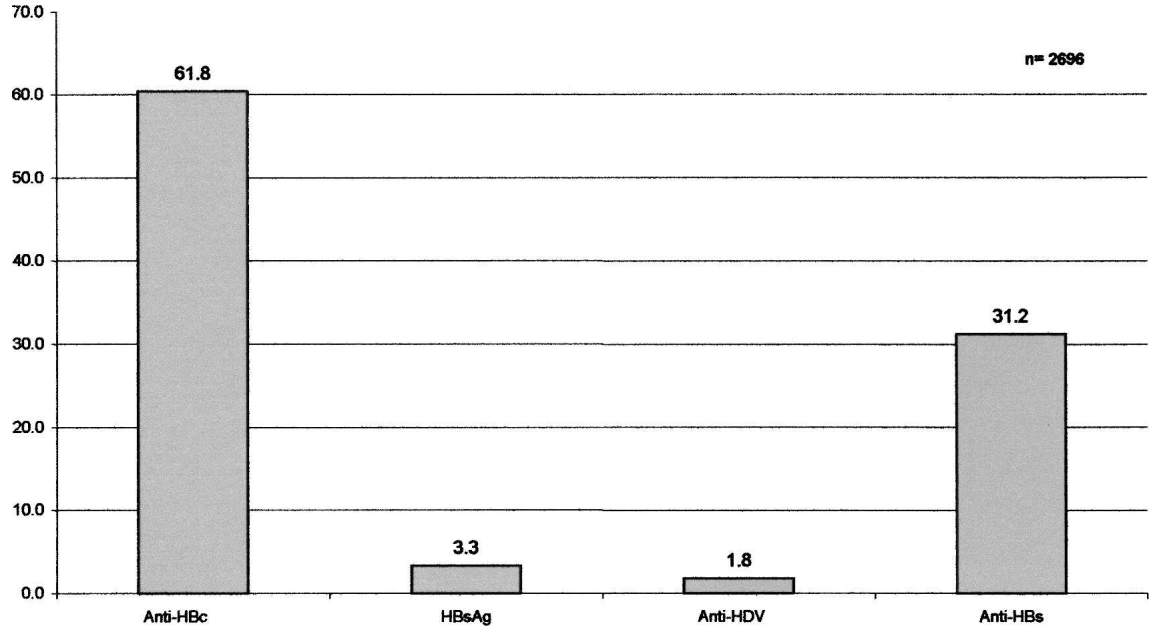

FIGURE 2. Prevalence (%) of antibodies to hepatitis B virus core antigen (HBc), hepatitis B virus surface antigen (HBsAg), antibodies to hepatitis D virus (HDV), and antibodies to HBsAg (HBs) detected in the population from 12 municipalities in the State of Acre, Brazil.

between municipalities and regions by waterways or air travel. These aspects favor the isolation of communities and, consequently, their diseases.[10]

Epidemiologic analysis indicates that HBV and HDV have been present in the region for a long time. The peculiarity that the HBV genotype F, which is prevalent in the Amerindian population of the region, as well as HDV genotype III, which is only found in this population, may indicate that these vi-

TABLE 2

Correlation between seropositivity for hepatitis delta virus (HDV) and sociodemographic characteristics of the population from 12 municipalities of the State of Acre, Brazil*

Variable	Anti-HDV n (%)				Chance ratio	
	Positive	Negative	Total	P†	Value	Limit
Sex						
Female	14 (1.3)	1,073 (98.7)	1,087	0.0002	3.146	1.7–5.9
Male	33 (3.9)	804 (96.1)	837			
Age group (years)						
0–9	1 (0.5)	181 (99.5)	182	NP[4]	NP	
10–19	5 (1.5)	322 (98.5)	327			
20–29	8 (1.8)	411 (98.2)	449			
30–49	21 (3.5)	584 (96.5)	605			
≥50	11 (3.5)	306 (96.5)	317			
Marital status‡						
Single	9 (2.0)	443 (98.0)	452	0.728	NP	
Married	28 (3.1)	890 (96.9)	918			
Widowed	2 (2.9)	66 (97.1)	68			
Separated§	2 (2.8)	69 (97.2)	71			
Place of birth						
Acre	39 (2.3)	1,694 (97.7)	1,733	0.838	1.819	0.8–3.9
Other state	8 (4.0)	191 (98.0)	199			
Rural area						
Already lived	31 (2.6)	1,150 (97.4)	1,181	0.491	0.808	0.4–1.5
Never lived	16 (2.1)	735 (97.9)	751			
Racial group						
Non-Indian	40 (2.2)	1,794 (97.8)	1,834	0.0019	3.45	1.5–7.9
Indian	7 (7.1)	91 (92.9)	98			
Educational level						
≤ 4 years	29 (4.1)	683 (95.9)	712	0.0023	2.763	1.3–5.5
≥ 5 years	12 (1.5)	781 (98.5)	793			

* NP = not performed.
† By chi-square test.
‡ In individuals ≥ 15 years old.
§ Separated or divorced.

TABLE 3
Possible risk factors associated with a positive serology for hepatitis delta virus (HDV) in 12 municipalities from the State of Acre, Brazil, 2003*

	Anti-HDV n (%)				Chance ratio	
	Reactive	Non-reactive	Total	P†	Value	Limits
Previous surgical procedures						
No	34 (2.5)	1,345 (97.5)	1,379	0.882	1.050	0.55–2.00
Yes	13 (2.4)	540 (97.6)	553			
Blood transfusions						
No	40 (2.2)	1,740 (97.8)	1,780	0.070	2.100	0.924–4.77
Yes	7 (4.6)	145 (95.4)	152			
History of malaria						
No	21 (1.8)	1,158 (98.2)	1,179	0.020	1.972	1.10–3.53
Yes	26 (3.5)	727 (96.5)	753			
Tattos						
No	41 (2.3)	1,781 (97.7)	1,822	0.034	2.506	1.04–6.04
Yes	6 (5.5)	104 (94.5)	110			
Hospitalizations						
No	23 (2.8)	793 (97.2)	816	0.886	1.320	0.74–2.35
Yes	24 (2.2)	1,092 (97.8)	1,116			
Tooth extractions						
No	11 (2.9)	366 (97.1)	377	0.496	1.268	0.64–2.51
Yes	36 (2.3)	1,519 (97.7)	1,555			
STD						
No	39 (2.2)	1,719 (97.8)	1,758	0.0520	2.124	0.97–4.62
Yes	8 (4.6)	166 (95.4)	174			
Use of injections						
No	23 (2.1)	1,092 (97.9)	1,115	0.217	1.437	0.80–2.56
Yes	24 (2.9)	793 (97.1)	817			

* STD = sexually transmitted diseases.
† By chi-square test.

ruses were introduced into the region before the migratory period and even before contact with Europeans and Asians.[15,16] Quintero and others[17] studied the distribution of HDV genotypes in Venezuela and confirmed the predominance of HDV genotype III among Amerindians and genotype I among non-Amerindians. This aspect deserves further study in Brazil.

A similar finding was observed in the high frequency of the F genotype of HBV in the Amerindian population of this region of Brazil. The present study confirmed the presence of the HBV genotype F in this region, which together with the A genotype, accounted for all cases. Unfortunately, we could only genotype 34 carriers of HBsAg. This low percentage of carriers for subsequent genotyping might be explained by the low viral load of asymptomatic carriers or conditions of serum transport and storage, which were highly precarious in some places. In addition, there is a paucity of studies on genotype F biomolecular assays. The primers used in our PCR assay may not have been adequate in amplifying this genotype. This could also explain the high proportion of samples positive for antibodies to HDV in isolated individuals who were also positive for antibodies to hepatitis C virus.

Serum samples from patients in this region must be collected under suitable conditions and tested for the presence of HBV DNA by a real-time PCR. This procedure can demonstrate the existence occult HBV infections in this area and/or point to the inadequacy of most PCR assays for genotype F. Also, the specificity of ELISAs for genotype F of HBV are lower because most are based on genotype A. The interpretation of the genotype distribution in different municipalities should consider the ethnic origin of the indigenous populations. Genotype F predominates in regions with a higher indigenous population, thus supporting the hypothesis that this genotype is peculiar to Amerindian populations.[18] Unfortunately, we were unable to amplify many samples. This resulted in an incomplete analysis of genotypes and ethnic aspects. As reported in the literature, comparison between subtypes and their respective genotypes showed a correlation, with subtype adw2 associated with genotype A and subtype adw4 with genotype F.[19]

One relevant peculiarity of the present study was the high frequency of HDV in the absence of HBsAg observed in 28 individuals. This result may indicate a past and resolved HDV infection.[20] Replication of HBV might have been suppressed by HDV to such an extent that serologic markers routinely used for the diagnosis of infection with HBV were undetectable.[21,22] This fact becomes even more relevant when one considers the possibility of infection with HDV genotype III. Cheng and others demonstrated peculiarities in the replication of this genotype.[23] One may speculate that genotype III of HDV interacts differently with HBV.

Ghuman and Kaur detected HDV markers in individuals without any HBV markers and suggested that the absence of HBV serologic markers is not sufficient to exclude infection with HDV because of the peculiar interaction between these two viruses.[24] If confirmed, this aspect should lead to changes in the screening policy of blood donors in the region because Brazilian regulations for blood donation do not include screening for HDV, which exposes individuals, especially patients with HBV, to the risk of transmitting this viral infection through blood transfusions.

Infection with the HDV was more prevalent among men and individuals with a low educational level. In addition, HDV was more frequent in older individuals. These findings suggest similar horizontal contamination routes for HBV and HDV in this region. By dealing with a virus related to agents causing diseases in plants,[25] the present study focused on the possibility that the forest environment is intimately associated with transmission of HDV. However, no association was observed between previous residence in a rural area of Acre and

the presence of antibodies to HDV. Nevertheless, the definition of urban and rural populations in the region is flawed, not only because the municipalities are scarcely populated, but also because many activities are performed in the forest even though the individual lives in the seat of the municipality, a fact that weakens analysis of this risk factor.

An association was observed between a history of malaria and jaundice and seroconversion to antibodies to HDV. Treatment with non-disposable syringes as previously described[12] for hepatitis C in Brazil[26] or diagnostic methods (use of a stylet for thick drop tests) were the contaminating routes in these individuals. Similarly, a higher frequency of seroconversion to antibodies to HDV was observed for previously tattooed individuals. It should be noted that there are reports showing a strong association between hepatitis C transmission and tattooing in Brazil and other countries.[27] A similar process can be expected for hepatitis B and D because both are viral infections transmitted by the parenteral route.

With respect to the habit of frequent mobility in forest areas (hunting, fishing, or camping), an association was observed with positive serologic findings for antibodies to HDV. This finding again raises the question whether HDV, or at least some of its genotypes, are directly related to the equatorial forest environment. The relevance of this hypothesis can be understood when one takes into account the peculiarities of HDV in the Amazon region, especially the occurrence of a particular form of fulminant hepatitis (black fever or Labrea hepatitis). This form of severe hepatitis was first described in the Brazilian Amazon region.[28] The first complete description of this enigmatic disease has been made (Santos B, unpublished data), and Bensabath and Dias[4] reported an association between this disease and infection with HDV. This type of fulminant hepatitis is characterized by liver failure even before hepatocellular necrosis and inflammation are observed. This aspect, together with the presence of morula cells (spongiocytes), makes this histopathologic picture peculiar and completely different from other forms of HDV fulminant hepatitis in which hepatocellular necrosis and inflammation predominate.

The occurrence of a similar disease also associated with HDV infection has been reported among natives from the African equatorial forest.[29] Despite efforts to explain this severe disease, only its epidemiologic relationship with tropical forest areas could be established.[13] The possibility of a higher pathogenicity of HDV genotype III, especially when associated with HBV genotype F, has been suggested by some investigators[30] who studied the disease in South America. In contrast, other investigators did not find the HDV genotype III in Africa but described mutations with the potential to modify viral pathogenicity.[31,32] Experimental transmission of this severe form of hepatitis to American woodchucks (*Marmota monax*), carriers of woodchuck hepatitis virus, supports the possibility that virologic aspects are the main cause of this particular type of fulminant hepatitis.[33] Other investigators have suggested new HDV genotypes in Africa.[34,35] Our group, in cooperation with a group in Lyon, France, is currently studying this aspect of HDV infection in the Brazilian Amazon region.

As long as these contradictory aspects remain, factors that may be related to the role of the forest environment in Labrea fever will continue to be investigated. Since hepatitis D is a disease that initially occurs in isolated tribal populations, this indicates the need for further epidemiologic studies involving indigenous populations of the region to better understand the transmission and occurrence of HDV among the different ethnic populations of the Western Amazon region.

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