EPIDEMIOLOGIC AND SEROLOGIC STUDIES OF ACUTE VIRAL HEPATITIS IN BRAZIL'S AMAZON BASIN[1]

Gilberta Bensabath,[2] Stephen C. Hadler,[3] M.C. Pereira Soares,[2] Howard Fields,[3] and James E. Maynard[2]

INTRODUCTION

Hepatitis has been identified as a major cause of morbidity and mortality in the Amazon Basin. Hepatitis morbidity may exceed 90 cases per 100,000 inhabitants per year in some parts of this region, and mortality from acute hepatitis on the order of four deaths per 100,000 inhabitants per year has been reported—a rate five to 10 times the average reported in the rest of the hemisphere *(1)*. Mortality due to cirrhosis is also reported as being high in some parts of the region. Furthermore, in rural parts of the southern Amazon tributaries an unusual type of severe hepatitis, known as Labrea hepatitis or black fever, has been recognized for 40 years *(2-5)*. This entity is characterized by rapid progression of acute hepatitis with hematemesis and/or encephalopathy developing within days of the onset of illness, together with a characteristic histologic picture of microvesicular fatty infiltration and eosinophilic necrosis of the liver *(5-6)*. It occurs in young adults and children, and often clusters in families. Recent studies have suggested combined hepatitis B virus (HBV) and delta virus infection as a possible cause of this illness *(7-8)*.

Several studies have indicated that both hepatitis A virus (HAV) and HBV infection are highly endemic throughout the Amazon region *(9-11)*. In addition, delta virus infection also appears to be endemic and to be a major cause of chronic hepatitis in this area *(8, 11)*. Nevertheless, studies to date have not focused upon the frequency and causes of acute and fulminant hepatitis in the Amazon region.

[1] This article will also be published in Spanish in the *Boletín de la Oficina Sanitaria Panamericana*, vol. 103, 1987. The work reported here was supported in part by a grant of FIPEC (Fundo de Incentivo a Pesquisa Técnico Científico do Banco do Brasil), FINEP (Financiadora de Estudos e Projetos of the Brazilian Ministry of Science and Technology), Polamazônia (Programa de Polos Agropecuários e Agrominerais da Amazônia), and by a Pan American Health Organization Fellowship to Dr Bensabath. Reprint requests may be directed to Dra. Gilberta Bensabath, Evandro Chagas Institute, Avenida Almirante Barroso 492-CP621, 66.000 Belém, Pará, Brazil or to Dr. Stephen Hadler, Hepatitis Branch, Bldg. 6, Room 154, Centers for Disease Control, NE, Atlanta, Georgia 30333, USA.

[2] Epidemiology Section, Evandro Chagas Institute, Fundação SESP (Serviços de Saúde Pública of the Brazilian Ministry of Health), Belém, Pará, Brazil.

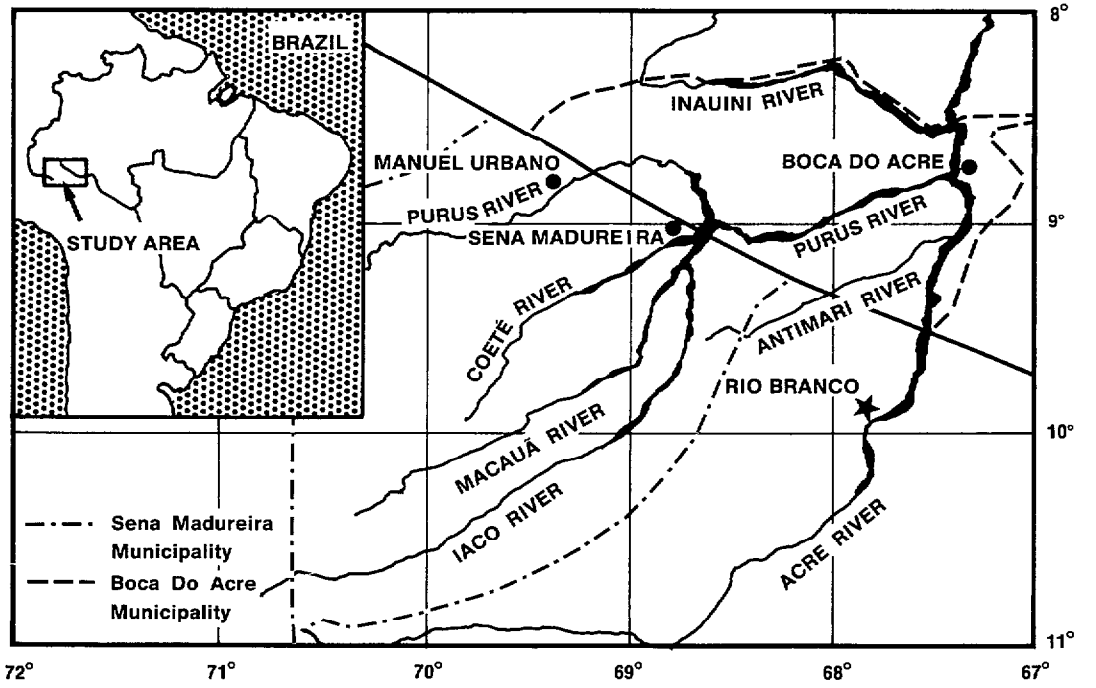
[3] Hepatitis Branch, Division of Viral Diseases, Centers for Disease Control, Atlanta, Georgia, USA.

MATERIALS AND METHODS

The findings reported here are based on a five-and-a-half year study of the causes of acute hepatitis in a district in the Purus River microregion of the southern Amazon Basin, a district well separated from the area's major cities.

Specifically, studies were conducted in the town of Boca do Acre (pop. 6,920 according to the 1980 census), in the municipality (district) with the same name (pop. 21,840 according to the 1980 census), and in the adjoining municipality of Sena Madureira, all of these sites being situated along the Purus River in the southwest part of the Amazon River Basin (Figure 1). The town of Boca do Acre, located at the confluence of the Acre and Purus rivers, possesses a 25-bed hospital (the only such facility in the district) staffed by two to four physicians. The town also has one public health post to provide services such as maternal and child health care. Homes in the town center are served by a chlorinated water system and use septic tanks for sewage disposal; however, dwellings at the town periphery or in the municipality's smaller villages usually use untreated river water and may not have systems for disposal of feces. The homes throughout the area usually have two or three rooms and are not protected against insects; the average number of people per dwelling is seven. The rainy season, when rainstorms occur daily, lasts from October through April;

FIGURE 1. A map showing the town of Boca do Acre and the study area municipalities of Boca do Acre and Sena Madureira, and their location in Brazil.



the average rainfall in this part of the year is 214.7 cm. The dry season occurs from May through September, and the average rainfall in this season is 64.3 cm.

Serum specimens used to assess the prevalences of HAV and HBV infections were collected in 1977–1979 from 1,017 individuals belonging to a random sample of 180 families in the town of Boca do Acre. The 464 people in the first 104 families tested were included in the study results reported here. Specimens were also collected from members of between 10 and 50 families in each of five villages with populations below 200 within the Boca do Acre and Sena Madureira municipalities.

From June 1979 to December 1984 a field office was operated in the town of Boca do Acre to conduct hepatitis surveillance and to collect serum specimens from cases of acute and fulminant hepatitis occurring within the municipality. Specimens were obtained during the first contact with the hepatitis case after diagnosis, usually within two weeks of disease onset. For purposes of this study, a case of acute hepatitis was considered to be one involving an acute onset of illness with jaundice and/or dark urine, together with aminotranferase test (ALT) results over two-and-a-half times normal and a negative blood smear for malaria. A diagnosis of fulminant hepatitis was made if the acute illness resulted in encephalopathy or death.

Serum specimens collected for seroprevalence studies were tested for markers of hepatitis A and hepatitis B infection. Those from the town of Boca do Acre were examined by enzyme immunoassays (EIA-Organon®) for total antibody to hepatitis A (anti-HAV), hepatitis B surface antigen (HBsAg), HBsAg antibody (anti-HBs), hepatitis B e antigen (HBeAg), and HBeAg antibody (anti-HBe). Sera collected from other parts of the two municipalities studied were tested by radioimmunoassays (RIA-Abbott®) for anti-HAV, HBsAg, anti-HBs, HBeAg, anti-HBe, and antibody to hepatitis B core antigen (anti-HBc). In both surveys, sera were considered HBsAg positive if they were strongly positive for HBsAg, irrespective of other markers. Study subjects in the town of Boca do Acre were considered immune if their sera were negative for HBsAg but positive for anti-HBs, while those in the other study settlements were considered immune if their sera were negative for HBsAg but strongly positive for anti-HBc or anti-HBs.

Serum specimens from acute hepatitis cases were initially tested by EIA-Organon® for HBsAg. All specimens negative for HBsAg were then tested for IgM anti-HAV, as were most (72%) of the specimens positive for HBsAg. All but three specimens negative for both HBsAg and IgM anti-HAV were then tested for IgM anti-HBc (EIA-Organon®). A case was classed as acute hepatitis A if the results were positive for IgM anti-HAV, as hepatitis A and B if the results were positive for both IgM anti-HAV and HBsAg, as hepatitis B if the results were positive for either HBsAg or IgM anti-HBc, and as non-A non-B hepatitis if the results were negative for IgM anti-HAV, HBsAg, and (if tested) IgM anti-HBc.

Specimens from cases of fulminant hepatitis were also tested for IgM anti-HBc (RIA-Abbott®), for total anti-delta by blocking EIA, for IgM anti-delta by u-chain-specific capture EIA, and for delta antigen by double antibody sandwich EIA after treatment with detergent *(12)*. Delta results are more fully discussed in a companion paper *(8)*.

The statistical significance of these various test results was assessed by the Chi-square test with Yates correction, or by Fisher's Exact Test where appropriate.

Results

Seroprevalences of HAV and HBV Infection

Testing for antibody to HAV indicated that this infection was acquired in early childhood in all the areas covered, but that infection occurred earlier in the larger town of Boca do Acre than in the relatively smaller villages (Table 1). In the town of Boca do Acre, only children under age 10 were found to be uninfected, with the prevalence rising rapidly from 46% among those under three years old to 84% among those five to nine years old and to 100% among all older subjects tested. In the smaller villages, the prevalence rose more slowly—from 14% among children under three years old to 96% among those between the ages of 15 and 19 years. The prevalence did not vary notably by sex in any area studied and varied only moderately (from 67% to 84%) in the different villages, but in each village the prevalence was lower than in the town of Boca do Acre.

The prevalence of hepatitis B was also high in all the survey areas. In contrast to hepatitis A, however, the prevalence of HBV infection was generally higher in the smaller villages than in the town of Boca do Acre (Tables 2 and 3). In the town of Boca do Acre, 6.9% of those tested were HBsAg positive, but the observed prevalence of HBV infection rose from 19% among children under five years old to 49% among children five to nine years old and to about 75% among those 10 years of age and older. In the smaller towns, a similar 6.3% of those tested were HBsAg positive overall, but the percentage varied markedly from town to town, from a low of 2.6% to a high of 10.4%. On the other hand, the

TABLE 1. Prevalences of antibodies to HAV found in sera from study subjects in different age groups residing in the town of Boca do Acre and other study villages. The last column indicates the statistical significance of differences between the results obtained from subjects in the town of Boca do Acre as compared to those in the other settlements.

	Study subjects residing in:				
	Boca do Acre (town)		Other settlements		
Age group (in years)	No. tested	% positive	No. tested	% positive	Statistical significance
0–2	24	45.8	58	13.8	$p < .01$
3–4	32	59.4	80	37.5	$0.5 < p < .10$
5–9	73	83.6	182	62.6	$p < .01$
10–14	80	100	134	80.6	$p < .01$
15–19	53	100	68	95.6	—
20–29	78	100	78	97.4	—
30–39	42	100	55	100	—
≥40	78	100	107	100	—
Total	460	91.7	762	73.9	$p < .001$

TABLE 2. The prevalences of HBsAg, anti-HBs, and overall HBV infection detected in the town of Boca do Acre, by age and sex.

	No. of subjects tested	Subjects positive for:				Total infected	
		HBsAg		Anti-HBs			
		No.	(%)	No.	(%)	No.	(%)
Age group (years):							
0–4	54	2	(3.7)	8	(15)	10	(19)
5–9	77	8	(10.4)	30	(39)	38	(49)
10–14	79	4	(5.1)	55	(70)	59	(75)
15–19	52	4	(7.7)	31	(60)	35	(67)
20–29	71	6	(8.5)	45	(63)	51	(72)
30–39	39	4	(10.3)	26	(67)	30	(77)
40–49	35	1	(2.9)	24	(69)	25	(71)
≥ 50	41	2	(4.9)	20	(49)	22	(54)
Sex:							
M	201	17	(8.5)	116	(58)	133	(66)
F	247	14	(5.7)	123	(50)	137	(55)
Total	448	31	(6.9)	239	(53)	270	(60)

TABLE 3. The prevalences of HBsAg, HBV immunity, and overall HBV infection in the study settlements outside the town of Boca do Acre, by age, sex, and settlement.

	No. of subjects tested	Subjects positive for HBsAg		Immune subjects (strongly positive for anti-HBs or anti-HBc)		Total infected	
		No.	(%)	No.	(%)	No.	(%)
Age group (years):							
0–4	138	9	(6.5)	33	(24)	42	(30)
5–9	182	7	(3.8)	102	(56)	109	(60)
10–14	133	14	(10.5)	93	(70)	107	(80)
15–19	69	5	(7.2)	59	(86)	64	(93)
20–29	78	5	(6.4)	70	(90)	75	(96)
30–39	55	5	(9.1)	48	(87)	53	(96)
40–49	51	1	(2.0)	48	(94)	49	(96)
≥ 50	57	2	(3.5)	54	(95)	56	(98)
Unknown	3	0	(—)	3	(100)	3	(100)
Sex:							
M	407	31	(7.6)	269	(66)	300	(74)
F	359	17	(4.7)	241	(67)	258	(72)
Settlement:							
Monte Verde	155	4	(2.6)	93	(60)	97	(63)
Boca do Coeté	216	16	(7.4)	127	(59)	143	(66)
Boca do Macauã	106	11	(10.4)	67	(63)	78	(74)
Seringão Maracaju	101	7	(6.9)	74	(73)	81	(80)
Praia dos Paus	188	10	(5.3)	149	(79)	159	(85)
Total	766	48	(6.3)	510	(67)	558	(73)

observed prevalence of HBV infection was less variable, and this prevalence tended to be higher than in the town of Boca do Acre, rising from 30% among those under five years old to 80% among those 10 to 14 and to over 90% among those 15 years of age and older. The prevalence of HBsAg was higher among men than among women in all the study areas, even though the overall prevalences of HBV infection differed minimally.

Among the detected HBsAg carriers, the observed prevalence of HBeAg was 49%. This latter prevalence did not differ notably by sex, but it varied markedly with age. That is, 88% of the study children under 10 years old who were positive for HBsAg were also positive for HBeAg. In contrast, only 44% of those children 10 to 14, 33% of those children 15 to 19, and 4% of the adults over 19 were HBeAg positive. Four of the 21 women of childbearing age (15–49 years) were HBeAg positive.

Acute Hepatitis

As Table 4 indicates, during the study period 401 cases of hepatitis (including 44 fulminant cases and 39 deaths) were diagnosed in the Boca do Acre Municipality. The overall rate of acute hepatitis was 3.33 cases per thousand inhabitants per year, almost 10 times the rates reported in North America and in some other parts of Brazil, and three times the rates reported in other parts of the Amazon region. The rate of fulminant hepatitis was 0.365 cases per thousand inhabitants per year, almost a hundred times higher than reported in most other parts of the Americas and ten times higher than reported in other parts of the Amazon.

Rates of acute hepatitis were found to be highest in children under age 10 and to decrease with increasing age. Overall, the rate found among women was 58% of that found among men. In general, the risk that an acute case would have a fulminant outcome was 11%, but this risk was highest (21%) among children 10 to 14 years; there appeared to be a somewhat higher risk of a fulminant outcome among men than among women (12% versus 8%), though this difference was not statistically significant.

The observed rate of acute hepatitis in the urban area (the town of Boca do Acre) was 5.6 cases per thousand inhabitants per year, a rate over twice as great as that found in the rural areas (Table 5). However, the observed rate of fulminant hepatitis was actually slightly higher in the rural areas. This suggests either that the illness tended to be more severe in the rural areas, or else (more probably) a tendency for the more severe rural cases to visit a town for treatment.

Using the aforementioned serologic criteria, 143 cases (37%) were diagnosed as hepatitis A, 16 (4.2%) as hepatitis A and B, 185 (48%) as hepatitis B, and 38 (10%) as non-A non-B hepatitis. Hepatitis A was most common in children under age 10, and was the predominant type in this group. Hepatitis B was frequent in all age groups, accounting for 40% of the hepatitis cases among children under age 10 and 60% of those among adults and older children. Non-A non-B hepatitis occurred primarily in adults and appeared to be the major cause of hepatitis among adults above age 40. No noteworthy differences were found in the rates at which different types of hepatitis occurred in the two sexes.

As Figure 2 shows, during the five-and-a-half year study period hepati-

TABLE 4. Observed cases of acute (including fulminant) hepatitis occurring in the municipality of Boca do Acre during the 1979–1984 study period, by the type of hepatitis and the patients' age and sex.

	No. of acute cases (total)	No. of fulminant cases	Rate of acute cases per 1,000 inhabitants per year	Type of hepatitis							
				A		A and B		B		Non-A non-B	
				No.	(%)[a]	No.	(%)[a]	No.	(%)[a]	No.	(%)[a]
Age group (years):											
0–4	108	10	4.43	61	(58)	4	(4)	41	(39)	0	(—)
5–9	110	8	5.51	47	(45)	9	(9)	45	(43)	4	(4)
10–14	52	11	3.25	15	(30)	1	(2)	30	(60)	4	(8)
15–19	38	4	3.06	11	(30)	1	(3)	21	(57)	4	(11)
20–29	44	4	2.54	6	(14)	1	(2)	28	(65)	8	(19)
30–39	23	1	2.01	2	(10)	0	(—)	13	(62)	6	(29)
≥ 40	19	2	1.03	0	(—)	0	(—)	6	(35)	11	(65)
Unknown	7	4		1	(—)	0	(—)	1	(—)	1	(—)
Sex:											
M	252	30	4.04	87	(36)	11	(5)	118	(49)	23	(10)
F	147	12	2.55	56	(39)	5	(3)	67	(47)	15	(10)
Unknown	2	2									
Fulminant cases		44	.365	0	(0)	2	(6)	27	(82)	4	(12)
All acute cases:	401		3.33	143	(37)	16	(4)	185	(48)	38	(10)

[a] The number of cases shown divided by the total number of cases affecting the relevant age group or sex whose type was diagnosed.

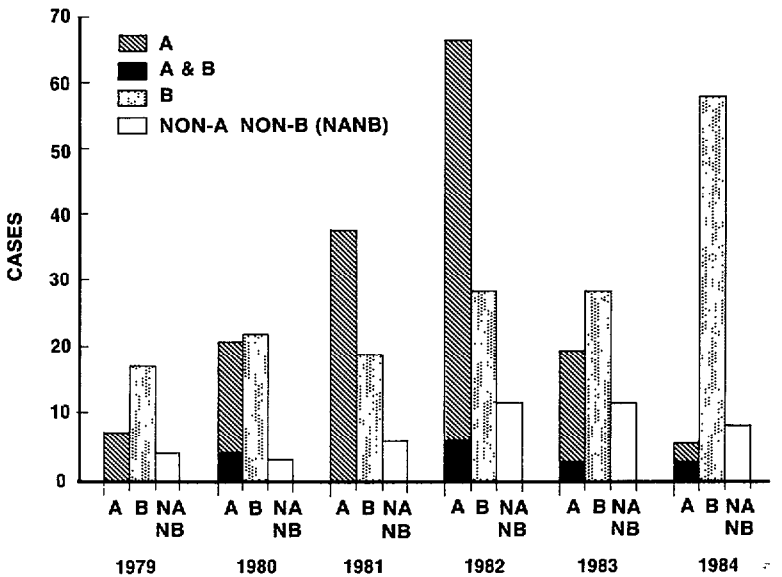
TABLE 5. The numbers and rates (per thousand inhabitants per year) of fulminant and nonfulminant acute hepatitis cases observed among residents of urban and rural areas of the municipality of Boca do Acre during the study period.

	No. of cases	Rate per 1,000 inhabitants per year
Fulminant cases:		
Urban	10	0.26
Rural	30	0.37
Nonfulminant cases:		
Urban	209	5.4
Rural	137	1.7
Total acute cases:		
Urban	219	5.6
Rural	167	2.1

tis A occurred in an epidemic cycle that peaked in 1982 with rates 10 times higher than those found at the beginning and end of the study. Seasonally, the peak months for hepatitis A cases were July to October, with disease rates being about 75% higher in the May-September dry season than in the October-April rainy season (P = 0.05). Hepatitis B appeared to be endemic, with rates varying less than twofold—except during the final year, when rates rose to three times the minimum levels. Non-A non-B hepatitis also showed a similar endemic pattern. No striking seasonality was found among hepatitis B cases or among non-A non-B cases.

Thirty-three of the 44 fulminant cases were tested serologically (see Table 4). Two were diagnosed as being cases of hepatitis A and B, four as cases of non-A non-B, and the remaining 27 (82%) as hepatitis B. Overall, the case-fatality rates were 6.5% for hepatitis A and B, 10% for non-A non-B, 12% for hepatitis B, and 0% for hepatitis A alone.

FIGURE 2. Acute hepatitis cases detected in the municipality of Boca do Acre during the study period (June 1979 through December 1984), by type of hepatitis and year.



Thirty-two of the fulminant hepatitis cases were tested for markers of delta infection in serum (and in liver, if a liver specimen was available). Among the 27 hepatitis B cases tested, 17 were strongly positive for delta infection, and three others gave a single weakly positive response. Because of an absence of IgM anti-HBc in the subjects' acute sera, most (89%) of these infections appeared to be delta superinfections of HBV carriers. Interestingly, one of three non-A non-B cases tested also showed delta antibodies. This person was positive for anti-HBc but negative for IgM anti-HBc, suggesting that delta superinfection of an HBV carrier with rapid loss of HBsAg provided a possible explanation for the development of fulminant hepatitis. In addition, both of the hepatitis A and B cases tested were also positive for delta antibodies, indicating the cause to be acute hepatitis A combined with HBV-delta infection. The observed mortality from hepatitis A combined with HBV and delta infection was significantly higher than that from hepatitis A alone ($P = .01$).

DISCUSSION AND CONCLUSIONS

This is the first population-based study of acute hepatitis in Brazil's Amazon Basin. It confirms previous surveillance data suggesting high rates of hepatitis in the region and suggests that rates of both acute and fulminant hepatitis there are much higher than previously suspected *(1)*. The major causes of the acute disease were found to be HAV and HBV infection. The importance of these viruses is confirmed by seroprevalence data showing uniformly high endemicity of both infections. Because other studies have shown comparably high endemicity of both viruses in other parts of the Brazilian Amazon, it is likely that both are also significant causes of acute disease throughout the region *(9–11)*.

In this area, hepatitis A was found to be mainly a clinical disease occurring in children under 10 years old. Despite a rapidly increasing prevalence of this infection during early childhood, the rates of clinical disease were high (over 100 cases per 100,000 inhabitants per year), and the virus was able to cause an epidemic similar to those observed in more developed areas where infection is normally delayed to later childhood. The number of cases in older children and young adults was also surprisingly high, given that almost all the seroprevalence study subjects over 14 years old showed immunity. Disease in these older people might be explained by high infection rates in the few people who survive early childhood without infection, or by in-migration of susceptible adults from areas where hepatitis A is less endemic.

Overall, hepatitis A infection alone was found to produce very low mortality; fulminant cases occurred only in persons who were HBsAg carriers and who also had delta infection. However, this study suggests acute HAV infection of an HBV-delta carrier may yield unusually high mortality. This finding differs from those of other studies suggesting that the severity of hepatitis A infection in HBV carriers does not differ from that of HAV infection alone *(13–14)*. In any case, the potential for high rates of acute disease would justify the development and use of a hepatitis A vaccine in this population.

Hepatitis B was the underlying cause of most hepatitis morbidity and mortality in the region. The sero-

prevalence studies showed a uniformly high HBV endemicity, with little variation from urban to rural settings, and with most of the population becoming infected during childhood. The peak prevalence observed in each study area was reached by late childhood, but a relatively lower proportion of adults showed infection in the relatively large settlement of Boca do Acre. This finding could be partly due to less complete and less sensitive serologic testing in the town; however, the lower prevalence of HBV infection observed among all younger age groups in this study suggests that the infection tends at least to be delayed to later childhood in the larger town.

Hepatitis B was found to be an important cause of acute hepatitis in both children and adults. It accounted for almost half the disease cases found among subjects under 15 years old—a proportion markedly higher than that reported from urban parts of Brazil and other South American countries *(1, 15–18)*. Unfortunately, it was not possible in this study to test all cases of acute hepatitis for IgM anti-HBc, the most specific indicator of recent HBV infection, or for delta infection. Thus, it is likely that some cases diagnosed as acute hepatitis B were actually due to infection of HBsAg carriers with other hepatitis viruses (delta virus or non-A non-B hepatitis). This circumstance is consistent with observation of a large number of children with acute HAV infection and concurrent acute or chronic HBV infection. Coinfection or superinfection with delta virus might help account for the unusual frequency of clinical HBV infection among children in this population, but further studies are needed to address this point.

The rates of fulminant hepatitis observed in the study region are among the highest documented and support the historical existence of an unusual type of fulminant hepatitis in this region. That HBV infection is the underlying "necessary" condition for this fulminant hepatitis is now undeniable; however, the other specific cofactors responsible for a fulminant outcome are still unclear. This study suggests a large proportion of the fulminant cases are due to delta virus superinfection of HBsAg carriers. Studies of isolated cases, as well as the similar reported characteristics of hepatic histopathology due to delta-superinfection among Venezuelan Indians and among persons living in the Santa Marta region of Colombia, support this concept *(7, 19, 20, 21)*. However, a significant proportion of the cases studied did not show delta virus infection, and a combination of causes (including hepatitis A in HBV-delta virus carriers, hepatitis B alone, and non-A non-B hepatitis, either alone or in HBV carriers) would be necessary to account for all of the cases. Whether all such entities coexist and produce a similar clinical and histopathologic picture, or whether current delta virus tests may not be sensitive enough to reliably detect all delta infections in fulminant disease cases is a matter that awaits further studies and development of more sensitive delta virus probes.

Acknowledgments

The authors wish to thank the following people for assistance in these studies: Guilherme Belgido Nunez, Lindomar Vasconcelos, and Carlos Alberto Tenorio of the Evandro Chagas Institute and Dr. José Oliveira Costa and the staff of the Health Services Hospital of Amazonas State for their help with the field

studies; Lynda Carter and Terri Nortol for preparation of the manuscript; Lois Conley, Nick de la Torre, Tracy Greene, and Barbara Shea for laboratory testing; and Dr. Francisco Pinheiro of the Pan American Health Organization for encouragement with the completion of this work.

Summary

To better define the incidence and causes of acute and fulminant hepatitis, the authors studied the prevalence and incidence of hepatitis A virus (HAV) and hepatitis B virus (HBV) infection in the area of Boca do Acre, a municipality in the extreme southwest of the Brazilian Amazon. Seroprevalence studies showed both hepatitis A and hepatitis B to be highly endemic throughout this region, with most people becoming infected within the first 10 years of life.

Hepatitis A caused 37% of all the observed cases, was the predominant type found among young children, and produced an epidemic disease pattern during a five-and-a-half year study period (from June 1979 to December 1984). Hepatitis B caused 48% of the cases and was a major cause of illness in both children and adults. Non-A non-B hepatitis occurred primarily in older adults.

During the study period the incidences of acute and fulminant hepatitis were 3.33 and 0.365 cases per thousand inhabitants per year. Over 85% of the fulminant hepatitis cases were found to involve active HBV infection, the patient's sera being positive for HBsAg. The single most common cause of fulminant hepatitis was delta virus superinfection of HBV carriers.

The observed rates of acute and fulminant hepatitis in Boca do Acre far exceed rates previously reported in the Western Hemisphere and in other parts of Brazil. While hepatitis A and hepatitis B are both important causes of acute hepatitis, combined HBV-delta virus infection is the major cause of the unusual type of fuminant hepatitis in this region.

References

1 Fay, O. H., S. C. Hadler, J. E. Maynard, and F. Pinheiro. Hepatitis in the Americas. *Epidemiological Bulletin, Pan American Health Organization* 6(5):1–7, 1985.

2 Boshell, J. M. Studies on the Amazonian Fever (Febre de Labrea; Febre Negra). In FSESP. *Belêm Virus Laboratory Annual Report*. Belém, Pará, Brazil, 1966.

3 DePaula, D., A. F. Pinheiro, L. B. Dias, and A. Sampaio de Lacerda. "Febre Negra" de Amazonia. *O Hospital* 71:123–131, 1967.

4 DePaula, D., A. J. Strano, and H. C. Hopps. Labrea hepatitis (black fever): A problem in geographic pathology. *International Pathology* 9:43–48, 1968.

5 Dias, L. B., and M. A. P. Moraes. Hepatite de Labrea. *Rev Inst Med Trop São Paulo* 15:86–93, 1973.

6 Bensabath, G., and L. B. Dias. Hepatite de Labrea e outras hepatitis fulminantes em Sena Madureira, Acre e Boca do Acre, Amazonas, Brasil. *Rev Inst Med Trop São Paulo* 25:182–194, 1983.

7 Fonseca, J. C. F., L. C. Gayotto, L. C. Ferreira, J. R. Araujo, W. D. Alecrim, R. T. M. Santos, J. P. Simonetti, and V. A. F. Alves. Labrea hepatitis—hepatitis B and delta antigen expression in liver tissue: Report of three autopsy cases. *Rev Inst Med Trop São Paulo* 27:224–227, 1985.

8 Bensabath, G., S. C. Hadler, M. C. Pereira Soares, H. Fields, L. B. Dias, H. Popper, and

J. E. Maynard. Hepatitis delta virus infection and Labrea hepatitis: Prevalence and role in fulminant hepatitis in the Amazon Basin. (Submitted for publication.)

9 Bensabath, G., and J. Boshell. Presença do antigeno Australia (Au) em populaçoes do interior do estado do Amazonas, Brasil. *Rev Inst Med Trop São Paulo* 15:284–288, 1973.

10 Gayotto, L. C. C., A. A. Quarentei, and G. L. Cabral. Seroepidemiology of hepatitis A and B in two areas of the West Amazon. *Gastroenterol Endoscopia Digestiva* 3:106–112, 1985.

11 Fonseca, J. C. F., J. P. Simonetti, L. C. L. Ferreira, I. K. Miranda Santos, A. Matos Tapares, and S. R. Simonetti. *Aspectos Peculiares da Hepatite no Brasil—Amazonia.* Instituto de Medicina Tropical de Manaus; Manaus, Brazil, 1984.

12 Fields, H. A., S. Govindarajan, H. S. Margolis, C. D. Schable, J. W. Ebert, and J. E. Maynard. Experimental transmission of the delta virus to a hepatitis B chronic carrier chimpanzee with the development of persistent delta carriage. *Am J Pathol* 122:308–314, 1986.

13 Hindman, S. H., J. E. Maynard, D. W. Bradley, K. R. Berquist, and A. E. Denes. Simultaneous infection with type A and B hepatitis viruses. *Am J Epidemiol* 105:135–139,1977.

14 Couteas, C., H. Kao, J. Rakela, and B. Weliky. Acute type A hepatitis in three patients with chronic HBV infection. *Digestive Diseases and Sciences* 28:684–686, 1983.

15 Ramonet, M., O. Arusa, N. Planes, M. Núñez, A. Afazani, and M. Ciocca. Etiología de la hepatitis viral aguda en una población pediátrica. *Boletín de la Sociedad Latinoamericana de Hepatología (Abstracts)* 5(2), 1984.

16 Koff, R. S., C. S. Pannuti, M. L. G. Pereira, B. G. Hansson, J. L. Dienstag, V. A. Weto, D. C. Wong, and R. H. Purcell. Hepatitis A and non-A non-B viral hepatitis in São Paulo, Brazil. *Hepatology* 2:445–448, 1982.

17 Velasco, M., E. Puelma, R. Katz, and J. Zacarias. Marcadores virales de la hepatitis aguda: Estudio en 291 niños y adultos en Chile. *Rev Med Chil* 110:542–546, 1982.

18 Zacarias, J., J. Rakela, M. Velasco, and P. Brinck. Prevalencia de los virus de hepatitis agudas en niños. *Rev Chil Pediatr* 54:316, 1983.

19 Hadler, S., M. Monzón, A. Ponzetto, D. Rivero, A. Mondolfi, A. Bracho, D. Francis, M. A. Gerber, S. Thung, J. Gerin, J. E. Maynard, H. Popper, and R. Purcell. Delta virus infection and severe hepitis: An epidemic in the Yucpa Indians of Venezuela. *Ann Intern Med* 100:339–344, 1984.

20 Popper, H., S. N. Thung, M. A. Gerber, S. C. Hadler, M. Monzón, A. Ponzetto, E. Anzola, D. Rivera, A. Mondolfi, A. Bracho, D. P. Francis, J. L. Gerin, J. E. Maynard, and R. H. Purcell. Histologic studies of severe delta agent infection in Venezuelan Indians. *Hepatology* 3:906–912, 1983.

21 Buitrago, B., H. Popper, S. C. Hadler, S. N. Thung, M. A. Gerber, R. H. Purcell, and J. E. Maynard. Specific histologic features of Santa Marta hepatitis: A severe form of hepatitis delta virus infection in northern South America. *Hepatology* 6(6):1285–1291, 1986.