Prevalence of hepatitis A virus infection: the paradoxical example of isolated communities in the western Brazilian Amazon region

Prevalência da infecção pelo vírus da hepatite A: o exemplo paradoxal de comunidades isoladas na região Amazônica Ocidental Brasileira

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ABSTRACT

This study evaluated the prevalence of hepatitis A virus infection in the rural area of Lábrea, in the western Brazilian Amazon region. Communities and households were selected randomly. Serum samples were analyzed by means of the immunoenzymatic method for the presence of total antibodies against HAV. The study included 1,499 individuals. The prevalence of anti-HAV was 74.6% (95% CI 72.3-76.8). Univariate analysis showed associations with age (chi-square for linear trend = 496.003, p < 0.001), presence of outside toilet (p < 0.001), history of hepatitis (p < 0.001) and family history of hepatitis (p = 0.05). After adjusting for age, HAV infection also showed an association with the number of people in the family (p = 0.03). The overall prevalence rates were high, but not more than 60% of the children under the age of ten years had already been infected. Very high prevalence was detected only within older cohorts, thus paradoxically defining this as a region with intermediate endemicity, even under the conditions of poverty encountered.

Key-words: Hepatitis A virus. Epidemiology. Prevalence. Amazon region. Brazil.

RESUMO

Este estudo avaliou a prevalência de infecção pelo vírus da hepatite A na área rural de Lábrea, Amazônia Ocidental Brasileira. Comunidades e domicílios foram selecionados aleatoriamente. Amostras de soro foram analisadas pelo método imunoenzimático para os anticorpos totais contra o vírus da hepatite A. O estudo incluiu 1.499 indivíduos. A prevalência do anti-HAV foi 74,6% (IC 95% 72,3 a 76,8). Análise univariada mostrou associação com idade (qui-quadrado de tendência linear = 496,003, p<0,001), presença de sanitário fora do domicílio (p<0,001), passado de hepatite (p<0,001) e história familiar de hepatite (p=0,05). Depois de controlado por idade, a infecção pelo VHA mostrou também associação com o número de pessoas na família (p=0,03). A prevalência global mostra taxas elevadas, entretanto não mais do que 60% dos menores de 10 anos, já são infectados, e prevalências elevadas são detectadas em coortes de mais idade, mostrando paradoxalmente uma definição de região de endemicidade intermediária, mesmo nas condições de pobreza encontradas.

Palavras-chaves: Vírus da hepatite A. Epidemiologia. Prevalência. Região amazônica. Brasil.

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Financial support: This study was funded by the Amazonas State Foundation for Support of Scientific Research (FAPEAM).

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Received in 26/11/2008
Accepted in 22/05/2009

Hepatitis A virus (HAV) distribution is associated with poor economic development, sanitation, environmental conditions and personal hygiene[9 11 19]. Three epidemiological patterns have been described according to the prevalence of past infection, as measured by the presence of total antibodies against HAV (anti-HAV). These patterns of prevalence are used because it is very difficult to estimate the incidence of this disease, due to the great number of asymptomatic cases, and because total anti-HAV is believed to be an immune marker that persists for years[25].

In areas of low endemicity, the overall prevalence is around 25%, outbreaks from a common source are very frequent, risk

groups can be defined and severe clinical cases are describe within old cohorts with an increased case fatality rate of 2%[19].

Areas of high endemicity have been described in developing and poor regions in which more than 90% of the population up to the age of 10 is already infected and the majority of the cases are asymptomatic[12].

Intermediate prevalence is said to be a transition stage between the high and low epidemiological patterns[9]. High prevalence rates of more than 90% can also be found mainly among young adults and adolescents, but in this situation, the rate of clinical disease may be higher and occurrences of severe clinical cases are very common[19].

In Brazil, HAV infection accounts for the majority of viral hepatitis notifications[24]. Changes in the epidemiological patterns have been described in some regions due to improvements in sanitation and socioeconomic conditions[10 22]. Nevertheless, all three patterns can be identified, even within a particular geographical region[7 24].

The present study had the aim of determining the prevalence of previous HAV infection among the populations of rural villages in the Purus river basin, within the municipality of Lábrea, in the western Brazilian Amazon region.

MATERIAL AND METHODS

The target population comprised the inhabitants of rural villages in the Purus river basin, within the municipality of Lábrea, in the western Brazilian Amazon region (**Figure 1**). Fifty-five villages were visited between March 2005 and April 2006, for data collection.

The study population was very homogeneous regarding living and environmental conditions. The villages are located along the banks of the Purus River. This area is flooded for half of the year and has very rich soil that is used for agriculture during the dry season. The villages are mostly inhabited by the families of former workers in the natural rubber plantations.

It is difficult to establish any social or economic differences, since all of the families seem to live at the same level of poverty and lack of environmental sanitation. Half of the population is illiterate. People earn money from selling of forest products, fish, beans and corn, and the income of the whole community in a village of one hundred inhabitants is no more than US$5,000 per year.

Nonetheless, very recently, we have noticed some improvements in terms of social programs settling people on the land. Most of the villages have a teacher living in the community. Rural properties have been transformed into natural reserves for sustainable management of natural resources, such as husbandry of wild animals like river turtles and some birds, gathering of forest products and exploitation of natural rubber.

This was a population-based cross-sectional survey. The sample size was estimated using the official rural area census population[13] and the estimated prevalence of antibodies against hepatitis C virus (anti-HCV) in the general population[21], since this study was also designed to evaluate the prevalence of serological markers for hepatitis B, C and D. Since the prevalence of anti-HCV is believed to be lower than that of the other types of hepatitis, the estimated sample size needed to be large enough to measure the prevalences of HAV, hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis delta virus (HDV). For sample calculation, we also used a design factor of 2, precision of ± 3% and 95% confidence level. These parameters yield a minimum sample of 1,287 individuals.

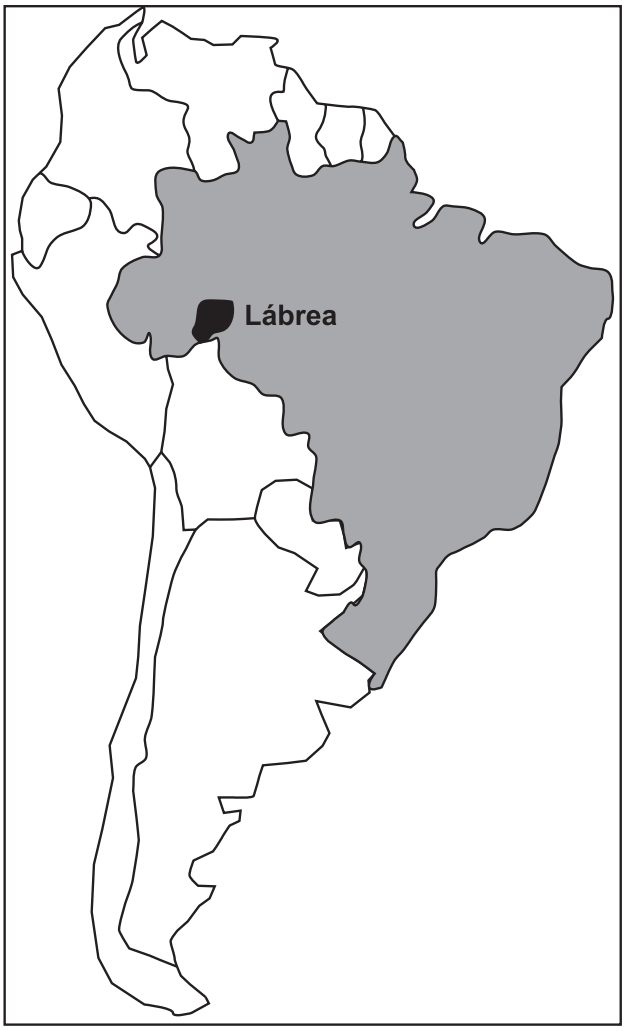

FIGURE 1

Map of the study area.

The villages were randomly selected from a list from the malaria control program, thus ensuring that we covered the whole geographical area of the municipality. Households were randomly selected after the research team arrived. In small villages of up to ten households, all the families were investigated. After subjects had given their written consent to their participation in the study, individual questionnaires were filled out and blood samples of 10ml each were taken from those present at the time of the investigation.

This study had previously been reviewed and approved by the Ethics Review Board of the Tropical Medicine Foundation of Amazonas (FMTAM), Manaus, Amazonas, Brazil.

Serum samples were analyzed by means of the enzyme-linked immunosorbent assay (ELISA) to test for total anti-HAV, using a commercial kit (DiaSorin, S.p.A., Saluggia, Italy). The test procedures followed the manufacturer's recommendations and were performed at the Virology Unit of FMTAM.

The anti-HAV prevalence and corresponding 95% confidence intervals (95% CI) were estimated in relation to the study variables of interest, such as gender, age group, water source, existence of treated drinking water, number of people per household, presence of outside toilet, parents' education level, history of clinical hepatitis and family history of hepatitis, among others. The chi-square test was used to compare proportions. Since age is positively associated with anti-HAV prevalence and is considered to be an important potential confounder, all statistical associations that were detected were controlled for using stratification by age group. Differences were considered significant with $p \leq 0.05$. The population was stratified by year for the first four years of life, and then by five-year increments up to the age of 19 and, finally, individuals aged 20 and over. Epi Info version 3.3.2[6] was used for statistical data management and analysis.

RESULTS

This study was conducted on a population of 298 families, totaling 1,499 individuals. The overall prevalence rate for total anti-HAV was 74.6% (95% CI 72.3-76.8). There was a heterogeneous pattern regarding the distribution between villages, ranging from 47.5% (29/61) in the village of "Amparo" (07°38'31.3"S; 65°28'25.3"W) to as high as 97.6% (40/41) in the village of "Praia do Gado" (07°16'52.8"S; 64°50'12.6"W).

Only one third of the subjects had already been infected by the age of five, and the proportion of immune subjects among the general population only reached 95% at the age of 20 and over. The age distribution of the study population is shown in **Figure 2**.

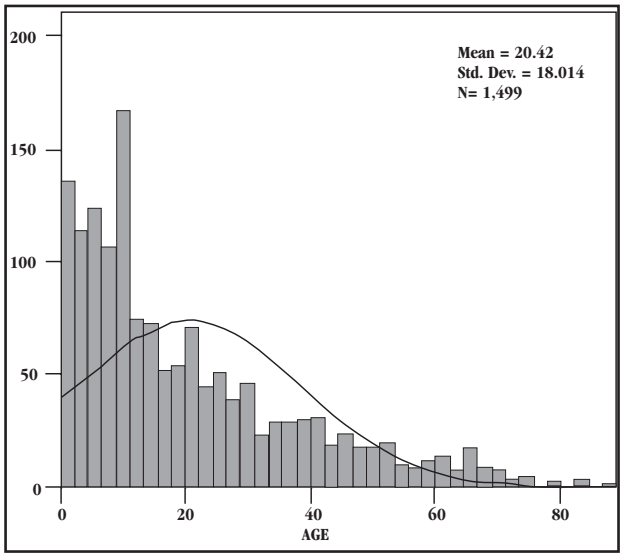

FIGURE 2

Age distribution of study population.

Univariate analysis showed that there was a statistical association with increasing age (chi-square for linear trend = 496,003, $p < 0.001$). Positive individuals were much older than the negative ones, with median ages of 20 and 5 years, respectively (Kruskal-Wallis test = 432,522, $p < 0.001$) (**Table 1**).

TABLE 1

Prevalence of total anti-HAV and distribution of associated risk factors among the rural population of the Purus River basin, municipality of Lábrea, State of Amazonas, Brazil.

Variable	Number	Number +	Anti-HAV IgG (%)	95% CI	*p*- value
Total sample	1,499	1,118	74.6	72.3 – 76.8	
Age group					
1y	65	21	32.3	30.3-34.3	
2y	71	9	12.7	10.5-14.9	
3y	47	16	34.0	31.7-36.3	
4y	67	23	34.3	32.4-36.2	< 0.0001*
5-9	296	178	60.1	59.4-60.8	
10-14	215	174	80.9	80.3-81.5	
15-19	140	125	89.3	88.8-89.8	
≥ 20	598	572	95.7	95.5-95.9	
Gender					
male	768	573	74.6	74.2-74.9	0.51
female	731	545	74.6	74.2-74.9	
Water source					
river	1,388	1,041	75.0	74.7-75.3	0.11
others**	111	77	69.4	68.4-70.4	
Treated drinking water					
yes	1,111	833	75.0	74.7-75.3	0.29
no	388	285	73.5	72.9-74.0	
Number of people per household					
≤ 5	635	478	75.3	74.0-75.7	0.32
> 5	864	640	74.1	73.7-74.4	
Outside toilet***					
yes	254	221	83.7	83.2-84.2	< 0.0001
no	1,235	897	72.6	72.3-72.8	
Parents' education****					
yes	88	20	22.7	20.9-24.4	0.13
no	162	49	30.2	29.4-31.0	
History of clinical hepatitis					
yes	260	244	93.8	93.5-94.1	
no	1,188	838	70.5	70.2-70.8	< 0.0001
Family history of hepatitis					
yes	975	742	76.1	75.8-76.4	0.05
no	483	348	72.0	71.5-72.5	

HAV: hepatitis A virus, +: number of individuals positive for anti-HAV IgG, 95% CI: 95% confidence interval, *p*-value: statistical significance, * chi-square for linear trend, ** wells and rain, *** outside of the household, **** among those < 5 years of age.

In addition, higher total anti-HAV was found to be associated with the presence of outside toilet facilities ($p < 0.001$), previous clinical history of hepatitis ($p < 0.001$) and family history of hepatitis ($p = 0.05$) (**Table 1**).

After adjustment by means of stratification, the risk of HAV infection remained positively associated with personal and family histories of clinical hepatitis and the presence of an outside toilet facility. On the other hand, we could also detect a significant association with the size of the family (**Table 2**).

TABLE 2

Unadjusted and age-adjusted associations with the presence of total anti-HAV and social demographic, environmental characteristics and past exposures, Lábrea municipality, Amazonas State, Brazil.

	Unadjusted			Adjusted		
Risk factor	RR	95% CI	p	RR	95% CI	p
Water supply	1.08	0.95-1.22	0.11	1.03	0.92-1.16	0.27
Treated water	1.02	0.95-1.09	0.29	1.01	0.95-1.07	0.32
Number of people per household	1.02	0.95-1.07	0.32	0.95	0.90-0.99	0.03
Parents' education	0.75	0.47-1.17	0.13	0.73	0.44-1.18	0.11
Past hepatitis	1.33	1.26-1.39	<0.001	1.09	1.05-1.18	<0.001
Family history	1.05	0.98-1.12	0.05	1.05	0.99-1.11	0.02
Outside toilet	1.15	1.08-1.22	<0.001	1.10	1.05-1.10	<0.001

RR: relative risk, 95% CI: 95% confidence interval, *p*-value: statistical significance.

DISCUSSION

Although the overall prevalence rates in the villages ranged from states of intermediate to high endemicity, no more than 60% of the whole population under the age of ten had already been infected. Prevalence rates of more than 90% were detected only in young adults, thus defining this region as one of intermediate endemicity, even under such conditions of poverty.

We concede that we had expected higher rates, since studies published previously had correlated poverty and poor sanitation with regions of high endemicity of HAV infection[5 13 16 26]. In addition, papers usually refer to northern Brazil as a very highly endemic region[3 7 15 20]. A prevalence rate of 93.7% was described among samples from Acre and the Purus river basin collected almost a decade ago[21].

Nevertheless, some epidemiological paradigms relating to HAV infection may be questioned, especially the positive association with rural areas and low income. Recently, a very heterogeneous scenario regarding HAV infection has been demonstrated, with rural areas showing infection rates lower than urban regions[1]. Furthermore, intermediate patterns have been described in isolated poor communities, just as we have described here[14].

In this specific study, poverty may be defined as economic abuse, but this population is living in circumstances of contact with huge quantities of natural resources from the forest, such as rich soil and plentiful fish. The communities are small but were settled not less than 20 years ago. Their children are culturally protected from environmental adversities through care such as the use of boiled water for food or washing, eat very well cooked food and lack of contact with the outside world for the first years. Differences in infection rates within villages may be due to small unmeasured variations in environmental sanitation and personal care.

The most important characteristic of regions with intermediate HAV infection patterns is the exposure of the population to HAV at older ages with increased morbidity[8 19]. Considering that these regions are highly endemic for HBV infection, especially among young adults[5], HAV could be contributing towards occurrences of severe clinical cases during superinfection of adolescents and young adults HBV carriers.

In situations in which there is a substantial risk of HAV infection at older ages, with important clinical outcomes, along with significant presence of HBV, HAV vaccination should be considered for inclusion in the immunization program for these specific populations, supported by a surveillance program for acute hepatitis.

Vaccination of programs focusing on adolescents in Catalonia have demonstrated efficiency in reducing the prevalence among all age groups under 60 years of age[8]. It has also been said that HAV could disappear from isolated communities through small improvements in sanitation and vaccination for susceptible individuals[10].

Surprisingly, among all of the sociodemographic factors evaluated, only the number of people living in the home, after adjustment, and the presence of an outside toilet facility showed statistical associations with positive tests for total anti-HAV.

Crowded homes are a well-known risk factor for HAV dissemination within families[19 23]. Indeed, the next-door and third and fourth houses away from the home of a positive case have been described as presenting increased risk of HAV infection[18].

It is quite odd to have to explain the presence of outside toilets as a risk factor for hepatitis A, but other studies have shown similar results in northeastern Brazil[1]. We believe that, since the soil is very wet and flooded, the few existing toilets (which are not properly built) actually feed the land and the neighbors' backyards with viruses and parasites, for instance after heavy rain. The properties have no fences and are linked by small bridges used during floods.

Transmission is probably facilitated by lack of sanitation, very wet soil and the climate. Nevertheless, individuals seem to be culturally protected until adolescence, when they have to start to challenge the forest and river for survival.

The possibility of change in HAV distribution patterns may be a matter for discussion, since prevalence rates of over 90% were described for this region, in a cohort of children aged up to 10 years, a decade ago[21]. Changes in epidemiological patterns are being described everywhere in developing countries[4 9 16 17 22], particularly within high social classes and in rural areas[2].

We believe that this question will be addressed a few years from now, since we have been studying a specific population. The

previous data was, in fact, based on a non-probabilistic sample of only 22 individuals up to the age of 10 years[21], and social programs had just been implemented.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the Municipality of Lábrea, through its mayor Gean Barros, and to the people of the villages we visited, for their very warm welcome and willingness to participate in this study. We are also grateful to Dr. Luiz Tadeu Figueiredo, for his peer review of this paper.

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