



## Article/Artigo

# Hepatitis B and C virus infection among Brazilian Amazon riparians

## Infecção pelo vírus da hepatite B e C em ribeirinhos da Amazônia brasileira

Claudia Suellen Ferro de Oliveira<sup>1</sup>, Adenilson Vilar e Silva<sup>1</sup>, Kemper Nunes dos Santos<sup>1</sup>, Amanda Alves Fecury<sup>1</sup>, Marcella Kelly Costa de Almeida<sup>1</sup>, Adriana Prado Fernandes<sup>1</sup>, Carlos Araújo da Costa<sup>1</sup>, Andrei Silva Freitas<sup>1</sup>, Tereza Cristina de Oliveira Corvelo<sup>2</sup> and Luisa Caricio Martins<sup>1</sup>

### ABSTRACT

**Introduction:** Viral hepatitis is a major public health concern in Brazil. There are few past studies on this issue, especially among riparian communities. This study aims at determining the seroprevalence of viral hepatitis B and C in the riparian community of Pacuí Island, within the Cametá municipality of Pará State, Brazil. Moreover, this study aims to investigate the principal risk factors that this community is exposed to. **Methods:** The current study has accessed blood samples from 181 volunteers who have answered an epidemiological questionnaire. Analyses on serological markers have been tested with commercial ELISA kits for detecting HBsAg, total anti-HBc, anti-HBs, and anti-HCV. Within seroreactive patients for HCV, RT-PCR and line probe assay have been performed to identify the viral genotype. **Results:** In the serological marker analysis for hepatitis B, no reactivity for HBsAg, rate of 1.1% for total anti-HBc, and rate of 19.3% for anti-HBs have been observed. On hepatitis C, 8.8% seroprevalence has been found, in which 62.5% have gotten viral RNA. Among the risk factors studied, the following have been highlighted: non-use of condoms, sharing of cutting instruments, use of illicit drugs, and reports of family disease with HBV or HCV. **Conclusions:** The vaccination coverage against HBV is low, and the high prevalence of HCV within this community has been observed.

**Keywords:** HBV. HCV. Amazon region. Riparians.

### RESUMO

**Introdução:** As hepatites virais constituem um importante problema de saúde pública no mundo. No Brasil existem poucos estudos sobre esta questão, especialmente entre as comunidades ribeirinhas. O objetivo deste estudo foi determinar a soroprevalência das hepatites B e C virais na comunidade ribeirinha da Ilha do Pacuí, no Estado do Pará, Brasil, e investigar os principais fatores de risco principal a que esta comunidade está exposta. **Métodos:** O presente estudo avaliou amostras de sangue de 181 voluntários que responderam a um questionário epidemiológico. Análises de marcadores sorológicos foram testados com kits comerciais de ELISA para detecção de HBsAg, anti-HBc total, anti-HBs e anti-HCV. Nos pacientes reagentes para HCV, RT-PCR e um *line probe assay* foi realizado para identificar o genótipo viral. **Resultados:** Na análise dos marcadores sorológicos para hepatite B, observou-se taxas de 1,1% para anti-HBc total e 19,3% para anti-HBs, o marcador sorológico HBsAg não foi encontrado nesta população. Para a hepatite C foi encontrada uma soroprevalência de 8,8%, destes 62,5% tinham RNA viral. Entre os fatores de risco estudados se destacaram: a não-utilização de preservativos, o compartilhamento de instrumentos cortantes, uso de drogas ilícitas e relatos de doença na família com VHB ou VHC. **Conclusões:** Observamos que a cobertura de vacinação contra o VHB é baixa e uma alta prevalência da hepatite C nesta comunidade.

**Keywords:** VHB. VHC. Região amazônica. População ribeirinha.

### INTRODUCTION

It may be reported that in Brazil, there is a major variation within the terms of prevalence of HBV and HCV, which is related to both groups studied and to the region itself. In the groups studied, the majority of information, regarding those forms of viral hepatitis, involves risk groups (patients on hemodialysis, health professionals, injectable drug users, prison inmates) or blood donors<sup>1-4</sup>.

Seroepidemiological studies related to HBV and HCV in indigenous people and riparians in the Brazilian Amazon are scarce due to access difficulties in those communities. Furthermore, the Amazon riparians have gotten their own features, mostly related to environmental, cultural, and socioeconomic factors<sup>1,5,6</sup>.

Notwithstanding the few studies carried out within the Amazon region, it is characterized as having a high rate of infection by HBV and intermediate rate to HCV and its consequences<sup>7,8</sup>.

The objective of this paper is to describe the seroepidemiological profile of viral hepatitis B and C in the Pacuí Riparian community, located in the Northern region, State of Pará, Brazil. The major risk factors to which the community is exposed are also analyzed to obtain knowledge about the epidemiology of these viruses within the population.

### METHODS

#### Site of the study

Pacuí is one of the 90 islands belonging to the Cametá municipality, located in the Northeast of Pará, Brazil. Pacuí is an island on the Tocantins River and is 180km from the state capital. It has an area of around 12.34km<sup>2</sup> and a population of 150 registered families, with 398 grown-up inhabitants according to the Municipality Health Secretariat (**Figure 1**). The island has two schools, two community agents, and no health center. Its economy is based on extraction and sale of regional fruits, as well as small-scale fishing. The residents live in wooden houses located along the river (**Figure 2**).

1. Núcleo de Medicina Tropical, Universidade Federal do Pará, Belém, PA. 2. Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, PA.

**Address to:** Dra. Luisa Caricio Martins. NMT/UFPA. Av. Generalíssimo Deodoro 92, Distrito de Umarizal, 66000-000 Belém, PA, Brasil.

Phone: 55 91 3201-6812

e-mail: caricio@ufpa.br

Received in 20/01/2011

Accepted in 25/04/2011

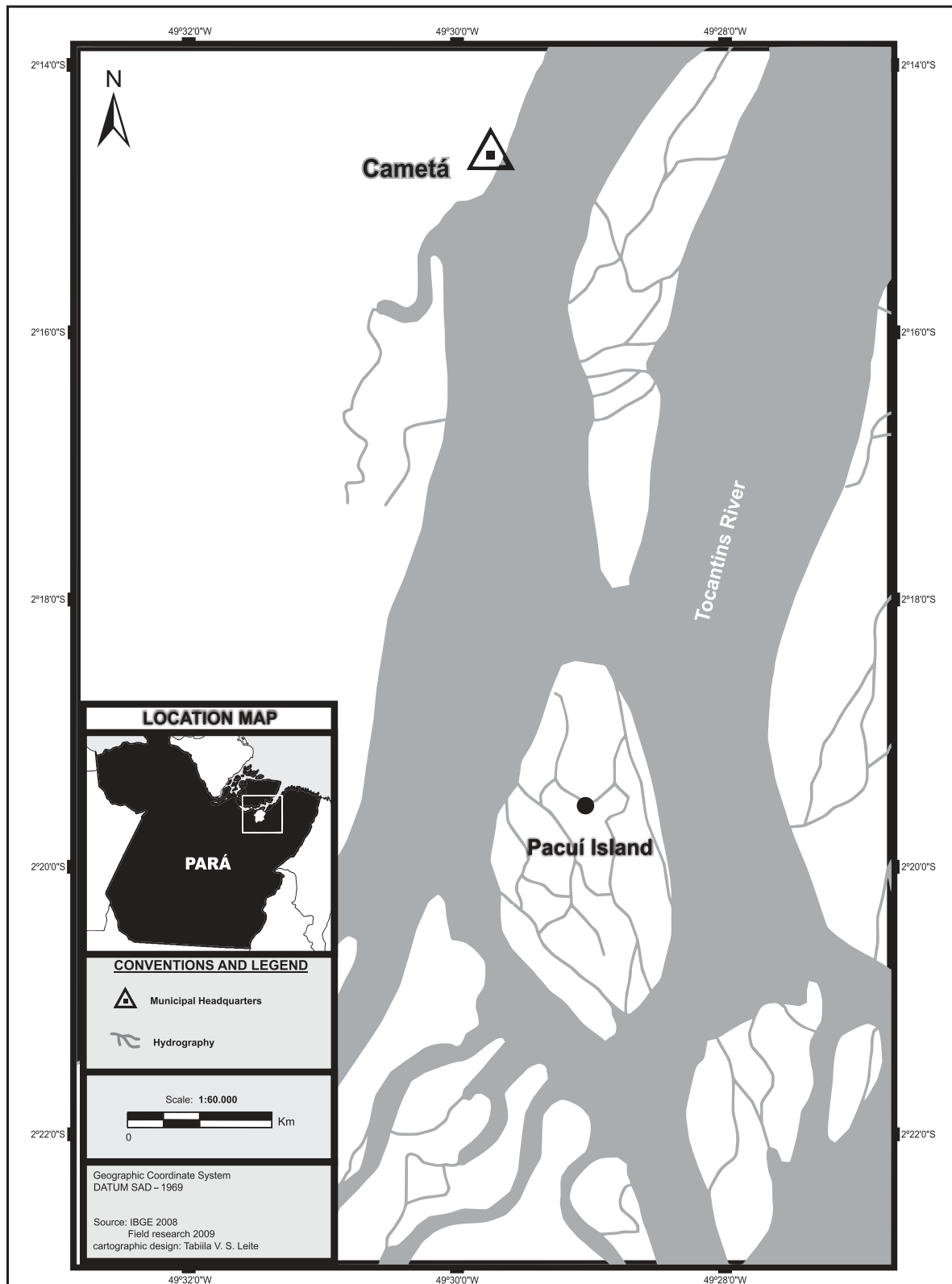


FIGURE 1 - Geographic location of Pacuí island, State of Pará, Brazil.



FIGURE 2 - Riparian's community house on Pacuí island, Cametá, Belém, State of Pará, Brazil.

### Patients and procedures

With support from the Municipality Health Secretariat and community agents, all adult inhabitants (18 years old or up) who live in the island and do not present any mental illness were made aware of the days that the team would be present and invited to take part in the study. From February 15-16, 2010, a professional health team collected biological samples and epidemiological forms from the residents in the island who agreed to take part in the study.

Based on spontaneous demand, 181 residents of Pacuí island who were 18 years old and above of both sexes took part in this study. They were from 80 families and comprised 45% of the grown-up island residents.

A standard and individual questionnaire was used to gather information about each patient, such as: age, sex, marital status, profession, education, family income, basic sanitation, and possible risk factors for transmitting viral hepatitis including the number of sexual partners within the last two years, use of condom, history of sexually transmitted diseases, blood transfusion, use of illicit drugs, shared cutting instruments, tattoo, and family history of HBV or HCV.

Approximately 8mL of blood were collected from each subject through peripheral venipuncture. The collected blood was centrifuged at 3,000rpm for 10 min, and the serum was separated and stored at -70°C for further analysis of viral markers.

### Serological and molecular diagnosis

The samples were tested to detect the markers of hepatitis B virus infection (HBsAg, anti-HBc, and anti-HBs) and were assessed by ELISA (Hepanostika Uni-form Organon Tecnica BV, Boxtel, Holland).

The presence of anti-HCV antibodies were tested using a third-generation enzyme immunoassay (ELISA) (Abbott Laboratories, Abbott Park, IL, USA). Positive samples were retested for confirmation using a line immunoassay (INNO-LIA HCV Ab III, Innogenetics). The entire samples were subjected to RNA extraction, reverse transcription, and a nested PCR with primers complementary to the conserved area of the 5' NC region of HCV, essentially as described by Ginabreda et al.<sup>9</sup> HCV genotyping was carried out for the entire HCV-RNA-positive samples. A line probe assay (Inno-LiPA HCV II, Innogenetics) was used to establish the genotype of the amplicons of the 5' NC region according to the procedure described by the manufacturer.

### Statistical analysis

The statistical program used was BioEstat 5.0<sup>10</sup>. The frequency distribution for the variables investigated was expressed in percentages, and the Chi-square test was used in comparing the proportions observed. To associate the risk factors with the serological markers, the *odds ratio* (OR) test was performed. The accepted statistical significance level was 95%.

### Ethical considerations

The current research has been approved by the Ethics Committee for Research with Human Beings at the Tropical Medicine Center (TMC), Belém, State of Pará, Brazil.

## RESULTS

The study was able to come up with a young population, with an age range of 32 years. The population characteristics were low level of schooling, predominantly female, 7% (12/181) were illiterate, 63% (114/181) had not finished elementary school, and 30% (55/181) already finished elementary school. Regarding the family income, 86% (156/181) were earning up to one minimum salary, and 14% (25/181) were earning two minimum salaries. The majority of the inhabitants in this community were earning their living from fishing and/or farming. The region had precarious basic sanitation, with 99% (179/181) of the interviewees using water from rivers and lakes and 97% (175/181) not having have septic tanks.

In serological marker analysis for hepatitis B, no HBsAg viral antigen was observed. As for the anti-HBs marker, it was found in 19.3% (35/181), and the anti-HBc was seen in 1.1% (2/181) of the individuals.

Only two (1%) individuals were anti-HBc- and anti-HBs-positive. Both were negative for IgM anti-HBc, indicating former infection with natural immunity.

Based on serological data for anti-HBs, low vaccination coverage against HBV was noted for the community, characterizing the inhabitants as a risk group for acquiring HBV.

The serological marker for hepatitis C (specific IgG HCV) was reactive in 8.8% (16/181) of the individuals (**Table 1**). Of those individuals, viral RNA was detected in 62.5% (10/16), and they all presented genotype 1 of HCV.

Regarding the risk factors, it was highlighted that 87% (157/181) of the volunteers were not using condom during sexual intercourse and 72% (131/181) were having a habit of sharing cutting instruments among relatives and/or neighbors.

When the risk factors were analyzed among the groups that were reactive for HCV with those who were not reactive, the reports showed that sexually transmitted diseases and the use of illicit drugs were more prevalent among those who were reactive (**Table 2**).

TABLE 1 - Prevalence of serological markers for hepatitis B virus and hepatitis C virus in the population studied.

Markers studied	Serological test	
	n	%
<b>Hepatitis B virus</b>		
HBsAg -; total anti-HBc -; anti-HBs - (susceptible)	146	81.0
HBsAg -; total anti-HBc +; anti-HBs+ (past infection)	2	1.0
HBsAg -; total anti-HBc -; anti-HBs + (vaccinated)	33	18.0
<b>Hepatitis C virus</b>		
anti-HCV	16	8.8

HBsAg: Hepatitis B surface antigen, Anti-HBc: Hepatitis B core antibody, Anti-HBs: Hepatitis B surface antibody, Anti-HCV: Hepatitis C antibody.

**TABLE 2 - Distribution of Hepatitis C virus serological marker and principal risk factors.**

Risk factors		Hepatitis C virus				OR (CI 95%)	p
		reactive		non-reactiven			
		n	%	n	%		
Age	≤30	5	31.2	57	34.5	0.86 (0.28-2.5)	0.9
	30	11	68.8	108	65.5		
Sex	female	9	56.5	106	64.2	0.71 (0.25-2.0)	0.7
	male	7	43.5	59	35.8		
Sexual partners in the last two years (n)	≤2	11	68.8	143	86.7	0.33 (0.10-1.0)	0.1
	≥3	5	31.2	22	13.3		
Use of condom	yes	1	6.25	23	13.9	0.41 (0.05-3.2)	0.6
	no	15	93.9	142	86.1		
History of STD	yes	11	68.8	19	11.5	16.9 (5.2-53.9)	<0.01
	no	5	31.2	146	88.5		
Blood transfusion	yes	2	12.5	19	11.5	1.0 (0.21-4.8)	0.7
	no	14	87.5	146	88.5		
Use of illicit drugs	yes	2	12.5	3	1.8	7.7 (1.1-50.1)	0.09
	no	14	87.5	162	98.2		
Nail shears shared	yes	13	81.2	118	71.5	1.7 (0.47-6.3)	0.5
	no	3	18.8	46	28.5		
Piercing/tattoo	yes	5	31.2	23	13.9	2.8 (0.89-8.8)	0.1
	no	11	68.8	142	86.1		
Family history of Hepatitis B virus	yes	2	12.5	2	1.2	11.6 (1.5-89.0)	< 0.05
	no	14	87.5	163	98.8		
Family history of Hepatitis C virus	yes	6	37.5	7	4.2	13.5 (3.8-47.9)	< 0.01
	no	10	62.5	158	95.8		

OR: Odds ratios, CI: confidence interval, STD: sexual transmitted diseases.

Furthermore, the reports showed that families with HBV or HCV infection were more prevalent among those who were positive (Table 2). Sixteen patients who were reactive for HCV were from eight families. In four families, two patients with HCV were found. There were two families with one patient, and two families had three patients.

## DISCUSSION

This is the first study regarding the prevalence of markers for viral hepatitis B and C in the Riparian community of Pacuí, Cametá municipality, State of Pará, Brazil.

The outcomes come out with a low prevalence of hepatitis B among the riparians investigated. The negative outcome observed for the HBsAg antigen earmarks the absence of hepatitis B in the population of the study. The presence of anti-HBc and anti-HBs markers in two individuals suggests that they have had contact with HBV. The concomitantly presenting anti-HBs and anti-HBc antibodies earmark post-hepatitis B immunity, probably a serological scar.

Notwithstanding, the presence of isolated anti-HBs marker in 18% (33/181) of the individuals has been noticed, which suggests a low rate of immunity acquired after vaccination. It is observed that the low level of vaccination reinforces the impression that this population is vulnerable to HBV infection. In Brazil, studies have demonstrated that infection with HBV is greater in 20- to 40-year-old individuals, which involve the sexually active population. Thus, vaccine coverage should be emphasized as the most rapid means of

dynamic socio-biological intervention against transmission in needy areas, such as the site of the present study<sup>6-8</sup>.

As for the marker for hepatitis C, a rate of 8.8% has been found for anti-HCV, which characterizes the community studied as a region of high prevalence for HCV.

Epidemiological research carried out among indigenous, riparian, and immigrant populations in the Brazilian Amazon has indicated that HBV seroprevalence may range from 0% to 20.6%, and for HCV, it may range from 0.4% to 4.8%<sup>3,5,7,11-13</sup>. Northern Brazil has the highest HCV seroprevalence (2.1%), with a high frequency of genotype 1<sup>14-16</sup>.

It is supposed that in Riparian communities, the prevalence of infections caused by HBV and HCV may be directly related to genetic, demographic, socioeconomic, cultural, and historical features among the population studied, as well as to other unknown factors<sup>13,17</sup>.

Regarding the comparison of the serological marker for HCV and the risk factors studied, this study has come up with the conclusion that the family environment is one of the principal reservoirs for HCV and, thus, contributes toward its dissemination and propagation.

Intrafamilial transmission of HCV is considered as a nonsignificant route of transmission, and sexual transmission does not seem to play a role in the intrafamilial spread of HCV infection<sup>18,19</sup>. However, some studies have demonstrated significant anti-HCV prevalence rates in family members of patients with chronic HCV and/or in household contacts of anti-HCV-positive individuals<sup>20</sup>. Although with low efficiency, sexual transmission should also be considered as one of the routes of intrafamilial transmission of HCV<sup>21</sup>.

Considering the high prevalence of HCV observed in this small community and the associated risk factors having been verified, further studies exploring these data should be carried out to confirm and assess the potential risk of family environment in the transmission of HCV infection.

HCV is primarily transmitted via parenteral routes. The use of illicit injectable or non-injectable drugs is currently considered as the most important risk factor for HCV<sup>21,22</sup>. This was also an important factor in our study.

For the population in this study, 72% of the individuals shared manicure tools among family members. The shared use of manicure and pedicure material should be taken into account by local and regional health authorities and by those of other countries with cultures similar to that of northern Brazil to establish political and public strategies to control HCV transmission.

The risk factors that have been verified are parenteral procedures that may be avoided if the population is made aware of the risks of transmitting microorganisms. Shaving or trimming nails may generate trauma or microtrauma on the skin surface, resulting in exposure to HCV on blades or nail scissors contaminated by asymptomatic infected family members.

In Italy, Pakistan, and Nigeria, HCV transmission by sharing razor blades and other cutting instruments as well as other forms of viral dissemination due to a lack of public awareness have been reported as risk factors for infection<sup>23-25</sup>.

Various transmission mechanisms have been studied in the Brazilian Amazon region; however, there some transmission factors that are yet to be determined<sup>26,27</sup>.

The high values for infection by HCV and the low vaccine coverage against HBV point to the need for preventive programs directed to this population at risk, which should be put in place based on knowledge of the epidemiological situation and transmission dynamics for those viruses.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## REFERENCES

- Ciorla LAS, Zanetta DMT. Hepatitis C within health care professionals: prevalence and association with risk factors. *Rev Saude Publica* 2007; 41:229-235.
- Marquesini G, Gonçalves NSL, Gonçalves Júnior FL. Prevalência dos marcadores sorológicos dos vírus da hepatite B (VHB) e da hepatite C (VHC) em hemodialisados. *Rev Pan Infectol* 2008; 10:23-27.
- Paraná R, Paiva T, Leite MR, Oliveira FN, Kali N, Lobato C, et al. Infection with hepatitis C virus amongst health workers in the Brazilian western Amazon Region (Rio Branco, State of Acre). *Am J Trop Med Hyg* 2007; 76:165-169.
- Lopes CL, Teles SA, Espírito-Santo MP, Lampre E, Rodrigues FP, Motta-Castro ARC, et al. Prevalence, risk factors and genotypes of hepatitis C virus infection amongst drug users, Central-Western Brazil. *Rev Saude Publica* 2009; 43:43-50.
- Ferreira CT, Silveira TR. Viral hepatitis: epidemiological and preventive aspects. *Rev Bras Epidemiol* 2004; 7:473-487.
- Aquino JA, Pegado KA, Barros LP, Machado LFA. Soroprevalência de infecções por vírus da hepatite B e vírus da hepatite C em indivíduos do Estado do Pará. *Rev Soc Bras Med Trop* 2008; 41:334-337.
- Paula VS, Arruda ME, Vitra LCL, Gaspar AMC. Seroprevalence of Viral Hepatitis in Riverine Communities from the Western Region of the Brazilian Amazon Basin. *Mem Inst Oswaldo Cruz* 2001; 96:1123-1128.
- Barros Júnior GM, Braga WSM, Oliveira CMC, Castilho MC, Araújo JR. Hepatite crônica B oculta: prevalência e aspectos clínicos em população de elevada endemicidade de infecção pelo vírus da hepatite B na Amazônia ocidental brasileira. *Rev Soc Bras Med Trop* 2008; 41:331-335.
- Ginabreda MGP, Yoshida CFT, Niel C. Genomic characterization of Brazilian hepatitis C virus genotypes 1a and 1b. *Braz J Med Biol Res* 1997; 30:339-345.
- Ayres M, Ayres Júnior M, Ayres DL, Santos AS. *BioEstat 5.0: Aplicações estatísticas nas áreas das ciências biológicas e médicas*. 5<sup>th</sup> ed. Belém (PA): Sociedade Civil Mamirauá. CNPq; 2007.
- Tavares-Neto J, Almeida D, Soares MC, Uchoa R, Viana S, Darub R, et al. Seroprevalence of hepatitis B and C in the Western Brazilian Amazon region (Rio Branco, Acre): a pilot study carried out during a hepatitis B vaccination program. *Braz J Infect Dis* 2004; 8:133-139.
- Khouri MEL, Duarte LS, Ribeiro RB, Silva LFF, Camargo LMA, et al. Seroprevalence of hepatitis B virus and hepatitis C virus in Monte Negro in the Brazilian Western Amazon Region. *Clinics* 2005; 60:29-36.
- Ferreira A, Greca D, Tavares E, Moriya Y, Spelling F, Boeira M, et al. Soroprevalência da hepatite B e C em índios Kaingang do Sul do Brasil. *Rev Panam Salud Publica* 2006; 20: 230-235.
- Grupo de Estudo da Sociedade Brasileira de Hepatologia. Epidemiologia da infecção pelo vírus da hepatite C no Brasil. *Gastroenterol End Digest* 1999; 18:S3-S8.
- Fonseca JCF, Brasil LM. Hepatitis C virus infection in the Amazon Brazilian region. *Rev Soc Bras Med Trop* 2004; 37:1-8.
- Campiotto S, Pinho JR, Carrilho FJ, Silva LC, Souto FJ, Spinelli V, et al. Geographic distribution of hepatitis C virus genotypes C in Brazil. *Braz J Med Biol Res* 2005; 38:41-49.
- Souto FJD, Santo GAE, Pietro BRC, Philippi JC, Azevedo RB, Gaspar AMC. Prevalência e fatores associados a marcadores do vírus da hepatite B em população rural do Brasil Central. *Rev Panam Salud Publica* 2001; 10:388-394.
- Hajiani E, Masjedizadeh R, Hashemi J, Azmi M, Rajabi T. Hepatitis C virus transmission and its risk factors within families of patients infected with hepatitis C virus within southern Iran: Khuzestan. *World J Gastroenterol* 2006; 12:7025-7028.
- Martins RM, Vanderborgh BO, Rouzere CD, Almeida VC, Cardoso DD, Yoshida CF. Low intrafamilial transmission of hepatitis C virus in central Brazil. *Rev Inst Med Trop Sao Paulo*. 1994; 36:547-549.
- Ackerman Z, Ackerman E, Paltiel O. Intrafamilial transmission of hepatitis C virus: a systematic review. *J Viral Hepat* 2000; 7:93-103.
- Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005; 5:558-567.
- Alter MJ. Epidemiology of hepatitis C virus infection. *World J Gastroenterol* 2007; 13:2436-2441.
- Bari A, Akhtar S, Rahbar MH, Luby SP. Risk factors for hepatitis C virus infection in male adults in Rawalpindi-Islamabad, Pakistan. *Trop Med Int Health* 2001; 6:732-738.
- Mele A, Corona R, Tosti ME, Palumbo F, Moiraghi A, Novaco F. Beauty treatments and risk of parenterally transmitted hepatitis. Results from the hepatitis surveillance system in Italy. *Scand J Infect Dis* 1995; 27:441-444.
- Koate BBD, Buseri FI, Jeremiah ZA. Seroprevalence of hepatitis C virus amongst blood donors in Rivers State, Nigeria. *Transfus Med* 2005; 15:449-451.
- Brasil LM, Fonseca JCF, Souza RB, Braga WSM, Toledo LM. Prevalência de marcadores para vírus da hepatite B em contatos domiciliares no Estado do Amazonas. *Rev Soc Bras Med Trop* 2003; 36:565-570.
- Cavalheiro NP, La Rosa A, Elagin S, Tengan FM, Araújo ESA, Barone AA. Hepatitis C: sexual or intrafamilial transmission? Epidemiological and phylogenetic analysis of hepatitis C virus in 24 infected couples. *Rev Soc Bras Med Trop* 2009; 42:239-244.