

## Hepatitis C Virus Prevalence among an Immigrant Community to the Southern Amazon, Brazil

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A community-based random survey was conducted in a southern Brazilian Amazonian county aiming to investigate hepatitis C virus (HCV) infection prevalence and the association of demographic variables and lifestyle behaviours. Seven hundred eighty individuals were serologically screened with a third generation enzyme-linked immunosorbent assay to detect anti-HCV antibodies between 1994/1995. Positive samples were retested for confirmation with a line immunoassay (LIA, Inno-LIA HCV Ab III). Most of these subjects were low income and came from southern Brazilian states (65.8). Two point four percent (IC 95% 1.2% - 4.6%) of the subjects had LIA-confirmed anti-HCV antibodies reactivity. The age-specific prevalence of HCV antibodies slightly increased with age, with the highest prevalence after the age of 40 years. The results of multivariate analysis indicate a strong association between HCV antibodies and previous surgery and history of intravenous drug use. There were no apparent association with gender, hepatitis B virus markers, blood transfusion, and sexual activity. Mean time living in Amazon did not differ between confirmed and negative anti-HCV individuals. The present data point out an intermediate endemicity of HCV infection among this immigrant community to the Amazon region and that few HCV infected participants presented known risk factors.

Key words: Amazon - emigration and immigration - epidemiology - hepatitis C virus

Hepatitis C virus (HCV) has been identified as the major causative agent of post-transfusion hepatitis (Alter et al. 1992). In addition to blood transfusion transmission, high prevalence of HCV antibodies has been detected among intravenous drug users. Other risk factors for HCV acquisition in the general population are the presence of tattoo/piercing, homosexual/heterosexual activity with increased number of partners and previous sexually transmitted diseases (STD). The efficiency of HCV transmission by these alternative routes of spread, other potential risk factors and co-factors such as concomitant HIV infection, are less known among nontransfused populations. Several studies have documented HCV vertical transmission although showing conflicting transmission rates (Mac Donald et al. 1996, Thomas et al. 1998).

There is a wide geographical variation of HCV prevalence in the general population (Choo et al.

1990, Contreras et al. 1991, Esteban et al. 1991, El-Sayed et al. 1996, Guadagnino et al. 1997). In Brazil data on HCV prevalence are mainly available from blood banks (Vanderborght et al. 1993, Martins et al. 1994, Silva et al. 1995). It is known that the Amazon region is highly endemic for hepatitis B virus (HBV) infection. Considering that HBV and HCV infections share similar modes of transmission, the investigation of seroprevalence rates and risk factors on HCV infection have public health importance.

Few publications have addressed HCV seroprevalence in the Amazon region and these studies were mainly conducted among selected groups, showing HCV prevalence ranging from 1.4% among Amazonian Indian tribes (Soares et al. 1994) to 3.6% among immigrants living in the region (Souto et al. 1996). However, interpretation of these seroprevalence studies should take into account the increased sensitivity and specificity of the different diagnostic assays performed in recent years compared to the first generation tests (MacDonald et al. 1996).

The present HCV community-based survey was conducted in a HBV moderate endemic area

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in a southern Amazonian county. The objectives were to investigate HCV infection and the association of demographic variables and lifestyle behaviours. These findings may allow to determine the endemicity of the region and to identify potential risk factors for viral transmission at populations level.

### MATERIALS AND METHODS

This analysis is based on data collected for a community-based random survey on HBV markers carried out in Terra Nova do Norte county (22,374 inhabitants) southern Brazilian Amazon region between December 1994 and February 1995. Details of the study design were presented previously (Souto et al. 1998a). Briefly, a survey was conducted in 275 houses randomly selected among 5,973 that were registered in the entire county. The calculated sample size was based on the target population of 16,289 inhabitants over the age of nine. Interviews were recorded during household visits and covered demographic characteristics, intravenous drug addiction, blood transfusion, hospitalization, sexual behaviour and malaria episodes. Previous HBV infectious was defined as any positive result of tested serum markers (anti-HBc, anti-HBs, HBsAg). Socio-economic status and migration were also evaluated through the family income and years living in the area. After the interview, a serum sample was collected and stored at  $-20^{\circ}\text{C}$ . Permission for carrying out the study was granted by regional health authorities and informed consent was obtained for all participants. The investigators guaranteed the confidentiality of the results. Medical counselling and follow-up were provided if necessary according to the serologic screening.

A third generation enzyme-linked immunosorbent assay (Elisa) was used to detect anti-HCV antibodies using a mixture core, NS3, NS4, and NS5 antigens (HBK 425-Hemobio, HCV Elisa, Embrabio, São Paulo, Brazil). Positive samples were retested for confirmation with a line immunoassay (LIA, Inno-LIA HCV Ab III, Innogenetics, Ghent, Belgium) using immunodominant epitopes of core, E2/NS1, NS3, NS4, and NS5 antigens). The tests were performed at Laboratory of Virology, Institute of Tropical Pathology, Federal University of Goiás, Goiânia, Brazil. HCV infection was considered present in individuals with a LIA-confirmed anti-HCV test.

Seroprevalence and 95% CI were calculated. Chi-square tests with Yates' correction or Fisher's exact test were performed to evaluate risk factors associated to HCV infection. Crude and adjusted odds ratio with 95% Confidence

Interval (95% CI) were calculated to determine the strength of association between variables in the uni and multivariate analysis using SAS 6.03 (SAS Institute, Cary, NC, US, 1988). Statistical significance was assessed at the 0.05 probability level in all analyses.

### RESULTS

Seven hundred eighty three subjects from 271 households were enrolled in the study. The participation rate was 92.6% and details of non-participation have been reported elsewhere (Souto et al. 1998a). Most of these subjects were Caucasian (55.5%) and came from southern Brazilian states (65.8%); 51.9% of the participants referred family income below or equal US\$ 2,500 annually. For 780 (99.6%) participants serum samples were available for testing HCV antibodies.

The overall prevalence of anti-HCV antibody by EIA was 4.3% (34/780). After testing by Inno-LIA 19 samples were confirmed positive for at least two antigens yielding a prevalence of 2.4% (95% CI 1.2-4.6). Only one subject was classified as indeterminate. The age-specific prevalence of HCV antibodies slightly increased with age, with the highest prevalence after the age of 40 years. Seroprevalence was similar between genders (Table I). Although this study was not designed to evaluate intra-familial cluster, there were only two HCV positives subjects living in the same household, suggesting no evidence of clustering in this population.

The results of univariate analysis indicate a strong association between HCV antibodies and previous surgery and history of intravenous drug use. There were no apparent associations with gender, HBV markers, blood transfusion, and sexual activity (Table I). Mean time living in Amazon did not differ between confirmed and negative anti-HCV individuals ( $t=0.19$ ;  $p=0.8$ ). Previous malaria episodes was slightly commoner among anti-HCV positive than in anti-HCV negative subjects. Finger prick for blood tests in order to performing malaria diagnosis was referred by 16 of 17 (94.1%) anti-HCV positive and by 580 of 720 (80.6%) anti-HCV negative, but with no statistical association ( $p=0.2$ ). Previous surgery and intravenous drug use remained significantly associated with anti-HCV, after adjustment for age and gender using a logistic regression model (Table I). Intravenous drug use and blood transfusion were referred for two and three of the 19 anti-HCV positive subjects, respectively. Despite of the questions about parenteral infection acquirement, this

TABLE I  
Demographic and risk factors associated with antibodies against hepatitis C virus in the Amazon region

Risk factor	Anti-HCV Positive/Total <sup>a</sup>	(%)	Crude OR (95% CI)	Adjusted OR <sup>c</sup> (95% CI)	p
Age group (years)					
10 – 19	3/226	(1.3)	1		0.6
20 – 29	5/172	(2.9)	2.2 (0.5-11.9) <sup>b</sup>	0.9	
30 – 39	3/152	(2)	1.5 (0.2-9.4)	(0.7;1.3)	
≥45	8/229	(2.6)	2.7 (0.6-13.0)		
Gender					
Female	9/375	(2.4)	1		0.4
Male	10/405	(2.5)	1 (0.4-2.8)	1.5 (0.5; 4.4)	
Previous IVD use					
No	16/752	(2.1)	1		<0.005
Yes	2/6	(33.3)	23 (2.7-163.6) <sup>b</sup>	17 (2.6; 108.8)	
Previous surgery					
No	9/563	(1.6)	1		<0.05
Yes	10/217	(4.6)	3 (1.1-8.1)	3.9 (1.3; 12)	
Blood transfusion					
No	16/701	(2.3)	1	-	-
Yes	3/72	(4.2)	1.9 (0.3-6.7) <sup>b</sup>		
Sexual activity					
No	13/502	(2.6)	1	-	-
Yes	6/273	(2.2)	1.2 (0.4-3.8) <sup>b</sup>		
Previous malaria episodes					
No	6/306	(2)	1	-	-
Yes	13/468	(2.8)	1.4 (0.5 – 4.6) <sup>b</sup>		
Finger prick for blood smear					
No	1/141	(0.7)	1	-	-
Yes	16/596	(2.7)	3.9 (0.6 – 163.1)		
Previous HBV infection					
No	6/353	(3.4)	1	-	-
Yes	13/427	(5.5)	1.8 (0.6-5.9)		

a: totals in Table may differ due to missing values; b: exact limits; c: logistic regression model including the variables gender, age (considered as a continuous variable), previous IVD use and previous surgery.

finding indicates that the study was not able to identify any HCV-associated classical risk factor in 15 (78.9%) of the anti-HCV positive subjects (Table II).

TABLE II

Potential transmission route among anti-hepatitis C virus positive subjects

Possible transmission route	n	%
Isolated injecting drug users	1	5.3
Isolated blood transfusion	2	10.5
Injecting drug users + blood transfusion	1	5.3
Sporadic occurrence <sup>a</sup>	14	78.9
Total	19	100

a: no classical risk factor or potential sexual exposure.

## DISCUSSION

This community-based survey shows 2.4% prevalence of confirmed anti-HCV in a county of southern Amazon. Previous studies showed prevalence ranging from 0% to 3.6% in the Brazilian Amazon (Souto et al. 1996, 1998b). The present study, with a larger and randomly chosen sample, yields a more representative result, indicating that this region has an intermediate endemicity of HCV infection. Prior surgery and intravenous drug use were consistently related to HCV infection as shown in logistic regression model. Probably, surgery represents HCV exposure beyond the own surgical procedure, through intravenous treatment, repeated blood drawing for examination, and transfusion of blood products. In fact, subjects undergoing surgery several years ago may be uninformed about, or simply forgotten whether blood products were received during prior hospitalization or surgical procedures. Intravenous drug use was closely associ-

ated with anti-HCV, suggesting that this is an important risk factor also in rural populations.

Surveys on HCV generally show that the infection occurs mainly as a cohort effect in subjects aged more than 45 years (Chiaromante et al. 1996, Diago et al. 1996). The use of non-disposable syringes and needles, a commonplace until two decades ago, is considered as a facilitating factor to HCV spread in some of these surveys (Chiaromante et al. 1996, Angelico et al. 1997). Common tropical diseases in Amazon, such as malaria and leishmaniasis, frequently need parenteral treatment and hospitalization. Finger prick for blood smears are required to diagnose malaria. This procedure was performed with non-disposable and non-sterilised cutting tools in the Amazon until few years ago. In our study, HCV positivity was not significantly associated with the variables involving diagnosis and treatment of malaria. Nonetheless, previous malaria as much as finger prick were more prevalent in HCV positive subjects, although not statistically significant, suggesting that these factors may have influenced the HCV transmission.

In this same community HBV infection occurred among 54.7% of the overall sampled population and the HBV subtypes detected were similar to the ones found at Brazilian southernmost regions, suggesting that the immigrants carried out HBV from their original states (Souto et al. 1998a). If the route of spread of HCV is similar to the HBV could not be determined by our present survey. Since the majority of HCV infection have a long latent period, the acquisition of infection may have occurred in the place of origin, before migration. In our study anti-HCV prevalence of the southern Brazilian immigrants did not differ from that observed in the other subjects. Furthermore, the mean time living in Amazon was not different between positive and negative anti-HCV subjects.

In conclusion, our data point out an intermediate endemicity of HCV infection in the Amazon region. Although, the present study has showed that few HCV infected individuals presented known risk factors, for the large majority the mode of transmission remained undefined in agreement with other studies in general population (Chiaromante et al. 1996, MacDonald et al. 1996, Angelico et al. 1997). Studies using more detailed-epidemiological questionnaires are needed in order to clarify this subject.

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