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Epidemiology of hepatitis B virus infection among recyclable waste collectors in central Brazil

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ABSTRACT

Introduction: The collection of recyclable waste materials is a widespread activity among the urban poor. Today, this occupation attracts an increasingly large number of individuals. Despite its economic and environmental importance, this activity is associated with unsafe and unhealthy working conditions. The aim of this study was to investigate the seroepidemiological profile of hepatitis B virus (HBV) infection in a population of recyclable waste collectors in central Brazil. **Methods:** Recyclable waste collectors from all 15 recycling cooperatives in Goiânia City were invited to participate in the study. The participants (n = 431) were interviewed and screened for hepatitis B surface antigen (HBsAg) and antibodies against HBsAg (anti-HBs) and hepatitis B core antigen (anti-HBc) by enzyme-linked immunosorbent assay (ELISA). HBsAg- and anti-HBc-positive samples were tested for HBV DNA and genotyped. **Results:** The overall prevalence of HBV infection (HBsAg- and/or anti-HBc-positive) was 12.8%. An age over 40 years and illicit drug use were associated with HBV infection. HBV DNA was detected in 2/3 HBsAg-positive samples and in 1/52 anti-HBc-positive/HBsAg-negative samples (an occult HBV infection rate of 1.9%), in which the genotypes/subgenotypes A/A1, D/D3 and F/F2 were identified. Only 12.3% of the recyclable waste collectors had serological evidence of previous HBV vaccination. **Conclusions:** These findings highlight the vulnerability of recyclable waste collectors to HBV infection and reinforce the importance of public health policies that address the health and safety of this socially vulnerable population.

Keywords: Hepatitis B. Recyclable waste collectors. Prevalence. Associated factors. Genotypes.

INTRODUCTION

Hepatitis B virus (HBV) infection represents the causative agent of global but preventable liver disease and remains an important public health problem, especially in developing countries. HBV infection can be transmitted through unprotected sexual intercourse, contact with contaminated blood or mother-to-child transmission, and this infection can lead to chronic liver disease, which affects more than 240 million individuals worldwide. Chronic carriers constitute a potential reservoir of HBV and are at increased risk of developing liver cirrhosis or hepatocellular carcinoma[1,2].

Hepatitis B surface antigen (HBsAg) represents an established serological marker for diagnosing acute or chronic HBV infection. HBV deoxyribonucleic acid (DNA) has, however, been detected in the serum and/or liver of individuals without detectable HBsAg levels. This peculiar form of chronic infection is referred to as an occult HBV infection and represents an HBV transmission risk, despite the low levels of detectable HBV DNA[3]. Based on sequence divergence of 8% or greater throughout the entire genome, HBV has been classified into 10 genotypes (A-J), which have defined geographic distributions[4].

The collection of recyclable waste materials is a widespread activity among the urban poor. In Brazil, selective waste collection is conducted by *catadores* (pickers), individuals who work either on an individual, informal and autonomous basis or in organized recycling cooperatives or associations. These pickers collect, separate, classify and sell all types of recyclable waste. In 2002, this job was regulated by the Brazilian Occupational Classification. Today, this occupation attracts an increasingly large number of individuals in urban areas. There are an estimated 1 million recyclable waste collectors in Brazil[5]. Despite its economic and environmental importance, this occupation is associated with unsafe and unhealthy working conditions. In addition, recyclable waste collectors have a lifestyle that is characterized by socioeconomic exclusion and stigmatization[6-12].

Occupational exposure to waste is a possible risk factor for HBV infection, and injury with needle sticks/sharp instruments could be associated with this infection[13]. A high prevalence of HBV infection (34.4%) was found among recyclable waste

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collectors in Santos City, Brazil[14,15], but no other data regarding HBV infection in this emerging urban population are available. Therefore, the aim of this study was to investigate the prevalence of and factors associated with HBV infection and to identify the genotypes of this virus circulating in a population of recyclable waste collectors in central Brazil.

METHODS

A cross-sectional study was performed in a population of recyclable waste collectors in Goiânia (a city with approximately 1,300,000 inhabitants), the capital of the State of Goiás, central Brazil. Since 2008, Goiânia has been engaged in the implementation of the *Programa Goiânia Coleta Seletiva* recycling program. This program focuses on integrating actions between collectors and the municipal government, which provides the collection of recyclables from every household. The rise in household recyclable collection has led to the creation of 15 cooperatives.

Between April 2010 and May 2011, recyclable waste pickers were recruited from all recycling cooperatives, and all individuals were invited to participate in this study. The study population consisted of 431 individuals. These participants represented 99.8% of the recyclable waste pickers who were participating in the cooperatives during the study period. Participation was voluntary. Written informed consent was obtained from all participants prior to the start of the study. The participants were interviewed to gather sociodemographic (age, gender, race/ethnicity, marital status, schooling and monthly income) and professional information and information on other risky behaviors (time spent as a recyclable waste collector, number of occupational accidents, history of blood transfusions, tattoos, illicit drug use, lifetime number of sexual partners, inconsistent or no condom use, incidence of previous sexually transmitted diseases [STDs] and incarceration) and their HBV vaccination status. All interviews were conducted in private locations at the participants' respective recycling cooperatives.

Blood was collected (10ml) from all participants, and serum samples were tested by enzyme-linked immunosorbent assay (ELISA) for the presence of HBsAg (Hepanostika HBsAg Ultra, bioMérieux, Boxtel, the Netherlands), antibodies against hepatitis B core antigen (anti-HBc) (Hepanostika anti-HBc Uni-Form, bioMérieux) and antibodies against HBsAg (anti-HBs) (Bioelisa anti-HBs, Biokit, Barcelona, Spain). HBsAg-positive samples were assayed for hepatitis B e antigen (HBeAg) and antibodies against HBeAg (anti-HBe) (Eti-Ab-Ebk Plus, Diasorin, Italy). DNA was extracted from HBsAg- and anti-HBc-positive samples as previously reported[16]. The pre-S/S genome region was amplified using a semi-nested polymerase chain reaction (PCR)[17], and the resulting amplicons were genotyped by nucleotide sequence analysis of the S region[18].

HBV prevalence was estimated using a 95% confidence interval (95% CI). Initially, a bivariate analysis was performed to determine the relationship between the dependent variable (the prevalence of HBV) and each independent variable, yielding prevalence ratios and respective 95% CIs. Variables with $p < 0.10$ were included in multivariate Poisson regression models. All analyses were performed using the Stata Statistical Package v. 11.

Ethical considerations

The protocol used in the present study was approved by the Ethical Committee of the Federal University of Goiás (Protocol Number: 002/2010).

RESULTS

The population of recyclable waste collectors studied herein was primarily composed of females (62.4%). The mean age was 36.9 ± 13.6 years. The majority of the participants were non-Caucasian (79.4%) and married (48.7%), had received nine or fewer years of formal education (elementary school in Brazil, 78.7%) and reported a monthly income of US$ 300 or less (52.7%).

Of 431 recyclable waste collectors, 55 (12.8%; 95% CI: 9.8-16.2) had been exposed to HBV (anti-HBc positive). Three (0.7%) of these individuals were HBV carriers (HBsAg positive). Forty-two had been infected and had developed natural immunity (anti-HBc and anti-HBs positive), and 10 were anti-HBc positive only. Fifty-three (12.3%) individuals were positive only for anti-HBs, suggesting that they had been vaccinated against HBV. In addition, most (74.9%) of the population studied was susceptible to HBV infection **(Table 1)**.

TABLE 1 - Prevalence of hepatitis B virus serological markers in 431 recyclable waste collectors in Goiânia-Goiás, central Brazil, 2010-2011.

HBV markers	Number	Percentage	95% CI
Any HBV exposure marker	55	12.8	(9.8-16.2)
Anti-HBc/HBsAg	3	0.7	(0.2-2.2)
Anti-HBc/anti-HBs	42	9.8	(7.2-12.8)
Anti-HBc only	10	2.3	(1.2-4.4)
Immunized (anti-HBs only)	53	12.3	(9.4-15.7)
Susceptible	323	74.9	(70.5-78.9)

95% CI: 95% confidence interval; HBV: hepatitis B virus; anti-HBc: antibodies against hepatitis B core antigen; HBsAg: hepatitis B surface antigen; anti-HBs: antibodies against HBsAg.

As shown in **Table 2**, an age over 40 years, a low schooling level, a history of incarceration and previous STDs were significantly associated with HBV infection ($p<0.05$). In addition to illicit drug use ($p=0.09$) and unprotected sex with multiple partners ($p=0.06$), these variables were included in a multivariate Poisson regression model. An age over 40 years and illicit drug use were independently associated with HBV infection.

TABLE 2 - Variables associated with hepatitis B virus infection in recyclable waste collectors in Goiânia-Goiás, central Brazil, 2010-2011.

Variables	HBV[a] prevalence (%)		PR (95% CI)	p value	Adjusted PR[c] (95% CI)	p value
Age (years)						
≤ 30	4/127	3.1	1.0		1.0	
31-40	10/107	9.3	2.96 (0.95-9.20)	0.06	2.98 (0.91-9.72)	0.07
41-50	13/68	19.1	6.06 (2.05-17.92)	< 0.01	6.18 (1.92-19.82)	< 0.01
51-60	18/55	32.7	10.39 (3.68-29.32)	< 0.01	10.07 (3.16-32.11)	< 0.01
> 60	10/21	47.6	15.12 (5.21-43.86)	< 0.01	16.09 (4.66-55.53)	< 0.01
Gender						
female	20/235	12.8	1.0		1.0	
male	25/143	17.5	1.37 (0.84-2.23)	0.21	0.79 (0.45-1.39)	0.43
Schooling (years)						
> 8	7/103	6.8	1.0		1.0	
4-8	19/156	12.2	1.80 (0.79-4.14)	0.16	1.75 (0.81-3.80)	0.16
< 4	29/119	24.4	3.58 (1.64-7.84)	< 0.01	1.75 (0.79-3.86)	0.17
Length of profession (years)						
≤ 1	27/195	13.8	1.0			
2-10	20/140	14.3	0.94 (0.55-1.62)	0.84		
> 10	8/43	18.6	1.29 (0.63-2.64)	0.48	-	-
Waste sharps/needle accidents[b]						
no	27/197	13.7	1.0			
yes	28/179	15.6	0.93 (0.57-1.53)	0.79	-	-
Blood transfusion[b]						
no	46/329	14.0	1.0			
yes	9/48	18.8	1.34 (0.70-2.56)	0.38	-	-
Tattoo						
no	43/307	14.0	1.0			
yes	12/71	16.9	1.2 (0.67-2.17)	0.53	-	-
Illicit drug use						
no	40/307	13.0	1.0		1.0	
yes	15/71	21.1	1.59 (0.93-2.72)	0.09	2.05 (1.15-3.68)	0.01
Incarceration[b]						
no	38/310	12.3	1.0		1,0	
yes	16/65	24.6	2.0 (1.19-3.37)	< 0.01	1.23 (0.69-2.22)	0.48
Unprotected sex with multiple partners (> 5)						
no	26/223	11.7	1.0		1.0	
yes	29/155	18.7	1.60 (0.98-2.61)	0.06	1.52 (0.87-2.67)	0.14
STDs[b]						
no	35/286	12.2	1.0		1.0	
yes	20/78	25.6	2.09 (1.28-3.42)	< 0.01	1.23 (0.76-2.02)	0.39

95% CI: 95% confidence interval; PR: prevalence ratio; HBV: hepatitis B virus; STDs: sexually transmitted diseases. [a]HBV-vaccinated individuals were excluded (n = 378). [b]The total represents the number of individuals who answered the question. [c]Adjusted for age, gender, schooling, history of illicit drug use, incarceration, unprotected sex with multiple partners and history of STDs.

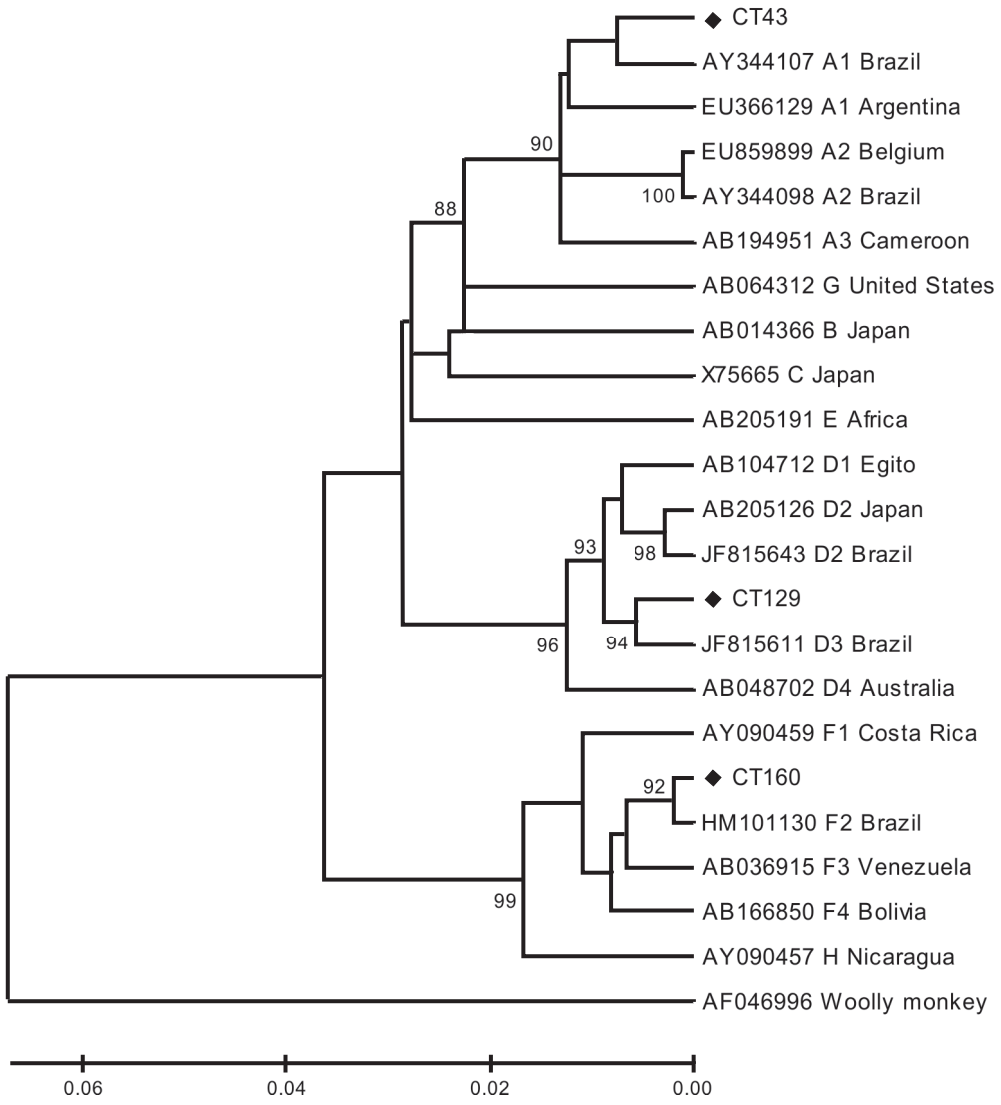

FIGURE 1 - Phylogenetic tree analysis based on nucleotide sequencing of the small S region of hepatitis B virus**.** The samples described in this study (◆CT 43, ◆CT 129 and ◆CT 160) and 19 representative strains of HBV genotypes (A-H) from GenBank, identified by accession number, genotype/subgenotype and country of origin, were included in the tree. The genome of woolly monkey HBV (GenBank AF046996) was utilized as an out-group. The tree was constructed using the neighbor-joining (NJ) method. The bootstrap values were calculated for 1,025 replicas, and consensus percentages are shown on the tree.

The three HBsAg-positive samples also possessed anti-HBe reactivity. Of these, two samples were positive for HBV DNA. Among the 52 HBsAg-negative/anti-HBc-positive samples (42 anti-HBc/anti-HBs and 10 anti-HBc only), one anti-HBc/anti-HBs-reactive sample was HBV DNA positive, resulting in an occult HBV infection rate of 1.9%. In the genotyping analysis, all three HBV DNA-positive samples could be sequenced in the S region. Using a phylogenetic tree analysis of the S region **(Figure 1)**, the HBV genotypes/subgenotypes A/A1, D/D3 and F/F2 were identified.

DISCUSSION

The prevalence of HBV infection found among recyclable waste collectors was 2.4-fold higher than that reported in a population-based study in the same region (5.3%; 95% CI: 4.6-6.1)[19]. Compared with the prevalence in other Brazilian populations, the prevalence determined in this study was within the confidence interval range recorded for patients attending an STD clinic (13%; 95% CI: 10.0-16.5)[20], non-injection drug users (14%; 95% CI: 11.7-16.5)[21] and individuals reporting to an HIV testing and counseling center (14.6%; 95% CI: 11.2-18.0)[22]. Nevertheless, relative to other reported data on recyclable waste collectors, the prevalence found in the present study was lower than that observed in Santos, Brazil (34.4%; 95% CI: 28.5-40.2)[14,15]. This difference may reflect differences in the rates of risky behaviors between the studied groups in Goiânia and Santos.

In the present study, an age over 40 years was significantly associated with HBV infection. As described elsewhere, age may be a proxy for lifetime exposure, indicating that over time, there

is a cumulative risk of acquiring an HBV infection linked to sexual and parenteral exposures[19,23]. This possibility is supported by the observation that illicit drug use was associated with increased HBV prevalence in the studied population. Among the 71 recyclable waste collectors who reported illicit drug use, the majority had used non-injection drugs only (88.7%; 63/71) (data not shown). Although the factors leading to an increased risk of hepatitis B in non-injection drug users are not well established, HBV infection is associated with unsafe sexual behavior and blood-borne transmission[21,24].

Other authors have reported that sexual transmission seems to be an important HBV transmission route in areas of low endemicity[19,25,26]. In the current study, however, unprotected sex with multiple partners and previous STDs were not significantly associated with HBV infection in the adjusted analysis, even though previous STDs were associated in the bivariate analysis. Moreover, unprotected sex with multiple partners was marginally associated. Risky sexual behaviors among recyclable waste collectors in Santos were also studied by Rozman et al.[15]. It should be stressed that most preventative policies related to STDs target groups with higher risks of exposure that do not include recyclable waste collectors.

A low schooling level and a history of incarceration were also associated with HBV infection in the bivariate analysis. As observed elsewhere, the level of education among recyclable waste collectors was low[7-9,12,15]. In fact, nearly 73% of the study population had less than 8 years of schooling. Those individuals who had been incarcerated also had limited educational opportunities and pervasive social health problems. These factors have been reported to be predictors of HBV infection and seem to be common in socially vulnerable populations[17,27-29].

Although occupational exposure to waste was not associated with HBV infection, a high frequency (47.6%; 179/376) of sharps and needle accidents was observed among recyclable waste collectors **(Table 2)**, as well as a low adherence to using gloves (63.6%) and other personal protective equipment (data not shown). These findings support previously reported data, reinforcing the importance of establishing educational and health policies that address the safety of this population[6,7,9,11,15].

The present study provides data describing the prevalence of occult HBV infection in recyclable waste collectors. Although no comparable data have been published, the proportion of occult HBV infection identified was similar to that reported in a community of African origin (1.7%), which utilized similar methods for the detection of HBV DNA[29]. The HBV genotypes/subtypes A/A1, D/D3 and F/F2 were identified in the three HBV DNA-positive serum samples, consistent with those circulating in the central-western region and other regions in Brazil[19,30,31]. Furthermore, it is noteworthy that the identified genotype F/subtype F2 may indicate movement of the population from the Amazon region, where the frequency of this genotype/subtype is considerable[30,32].

In addition to expanding hepatitis B vaccination, only 12.3% of the study population had a serological profile indicative of previous HBV vaccination, and 74.9% were susceptible to HBV infection. In addition, it is worth noting that only 29.7% (27/91) of the recyclable waste collectors who were eligible for free hepatitis B vaccination in Brazil (during the study period, all individuals under 24 years of age) had serological evidence of vaccination (data not shown), indicating low hepatitis B immunization coverage. The Brazilian Immunization Program recommends the administration of the hepatitis B vaccine to newborns, children and, more recently, adults under 50 years of age as well as to high-risk individuals, not including recyclable waste collectors.

These results must be considered in the context of the study's limitations. This was a cross-sectional study, and the behavioral findings were based on self-reports. Although this study included 99.8% of the recyclable waste pickers who were participating in all recycling cooperatives in Goiânia City, central Brazil, this group does not represent all recyclable waste pickers in this city. Moreover, given that there is a lack of available data on the epidemiology of HBV infection among recyclable waste pickers in central Brazil, these data provide valuable insight into this topic.

In conclusion, this study revealed that the HBV prevalence found among recyclable waste collectors was higher than that reported in a population-based study in the same region. In addition, the low vaccination rates and the presence of risky behaviors found in the study population highlight a need for improved health awareness and hepatitis B prevention programs in recycling cooperatives in central Brazil.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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