



## High level of exposure to hepatitis B virus infection in a vulnerable population of a low endemic area: A challenge for vaccination coverage★



Sabrina Moreira dos Santos Weis-Torres<sup>a,\*</sup>, Sonia Maria Fernandes Fitts<sup>a</sup>, Wesley Márcio Cardoso<sup>a</sup>, Minoru German Higa Junior<sup>a</sup>, Lívia Alves Lima<sup>a</sup>, Larissa Melo Bandeira<sup>a</sup>, Vivianne Oliveira Landgraf Castro<sup>a</sup>, Fátima Aparecida Carneiro<sup>b</sup>, Luciana Maria Marangoni Iglecias<sup>a</sup>, Gabriela Alves Cesar<sup>a</sup>, Tayana Serpa Ortiz Tanaka<sup>a</sup>, Marco Antonio Moreira Puga<sup>a</sup>, Grazielli Rocha Rezende<sup>a</sup>, Julio Croda<sup>a,b</sup>, Bárbara Vieira Lago<sup>c,\*\*</sup>, Ana Rita Coimbra Motta-Castro<sup>a,b</sup>

<sup>a</sup> Federal University of Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil

<sup>b</sup> Oswaldo Cruz Foundation, Campo Grande, Mato Grosso do Sul, Brazil

<sup>c</sup> Oswaldo Cruz Foundation, Rio de Janeiro, Rio de Janeiro, Brazil

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### ABSTRACT

**Objectives:** To evaluate the epidemiological and molecular features of HBV infection among recyclable waste collectors.

**Methods:** The participants were recruited from the dumping ground and recycling cooperatives in Campo Grande, Brazil, and were screened for hepatitis B and C, and HIV serological markers by ELISA, confirmed by PCR.

**Results:** Among 278 participants, 63.7% (95% CI: 58.0–69.3) were considered susceptible to HBV infection. The prevalence rate of HBV exposure was 10.1% (95% CI: 6.5–13.6) and 0.4% (95% CI: 0.1–0.6) were chronic carriers. Age  $\geq 45$  years (AOR = 7.15), history of homosexual contact (AOR = 5.29), tattoo (AOR = 4.92) and history of surgery (AOR = 2.89) were factors associated with ever infection. Age 18–25 years (AOR = 4.63), educational level  $\geq 9$  years (AOR = 1.98) and knowledge about HBV transmission (AOR = 3.08) were associated with serological HBV vaccination like profile (26.2%; 95% CI: 21.1–31.4).

**Conclusions:** Despite the availability and efficacy of HBV vaccine, this study found high HBV exposure and proportion of susceptible adults in a low endemic area. Vaccination and screening campaigns using accessible language to the economically disadvantaged populations can reduce the number of people susceptible to HBV infection.

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### Introduction

In recent decades, there has been a decrease in the prevalence of hepatitis B virus (HBV) infection in the central region of Brazil due to vaccination coverage, especially among young people and children (Pereira et al., 2009; Lindenberg et al., 2013; Souto, 2016). Despite this, several studies conducted in the same region have shown a high prevalence of HBV infection in vulnerable populations, including prisoners, drug users, truck drivers, homosexual men and recyclable waste collectors (Seage et al., 1997; Tavares-Neto et al., 2004; Neaigus et al., 2007; Nunes et al., 2007; Matos et al., 2008; Rozman et al., 2008; Stief et al., 2010; Freitas et al., 2014; Iglecias et al., 2016).

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\* Corresponding author at: Federal University of Mato Grosso do Sul, Avenida Senador Filinto Müller, s/n, Laboratório de Imunologia Clínica, FAFAN, 79070-900, Campo Grande, MS, Brazil and laboratório de hepatites Virais, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, 21040-360, Rio de Janeiro, Brasil

\*\* Corresponding author.

E-mail addresses: [weistorres.sms@gmail.com](mailto:weistorres.sms@gmail.com) (S.M.d.S. Weis-Torres), [barbaravilago@gmail.com](mailto:barbaravilago@gmail.com) (B.V. Lago).

Recyclable waste collectors are at high risk of acquiring numerous infections due to their exposure to several occupational, environmental and behavioral factors related to this activity. They collect, separate, classify and sell all kinds of recyclable waste, living on the margin of society and facing a lack of perspective, unemployment, and vulnerability. This socioeconomic exclusion and stigmatization (Rozman et al., 2008; Porta et al., 2009), combined with risky behaviors, lead to a high vulnerability to infectious diseases (Medeiros and Macêdo, 2006; Marinho et al., 2014; Trépo et al., 2014). HIV, HBV and hepatitis C virus (HCV) are most commonly transmitted through percutaneous, parenteral and sexual contacts and may be present in several types of solid waste: tissues, dressings, preservatives, absorbents, needles, and syringes (Liaw and Chu, 2009; Trépo et al., 2014). In addition, accidents with sharp material expose these professionals to a greater risk for the acquisition of infectious diseases transmitted by blood and/or secretions (Porta et al., 2009).

To our knowledge, there are few studies worldwide regarding the epidemiological status of HBV infection and factors associated with hepatitis B immunization among recyclable waste collectors (Rozman et al., 2008; Marinho et al., 2014). Therefore, this study aims to characterize the epidemiological and molecular aspects of HBV infection among recyclable waste collectors in Campo Grande, Central Brazil.

## Material and methods

A cross-sectional survey was conducted among recyclable waste collectors in Campo Grande, the capital of Mato Grosso do Sul state, Central Brazil. Between April 2014 and July 2016, the participants were recruited (convenience sample) from the dumping ground and from all six recycling cooperatives. The study population included male and female subjects aged 18 years or older who work either on an individual, informal and autonomous basis or in organized recycling cooperatives. The required sample size was estimated in 121 participants, based on expected 12.8% of HBV prevalence among recyclable waste collectors (Marinho et al., 2014), considering 95% CI, 5% margin error, and 20% non-respondent.

The individuals were informed in detail about the research objectives and the confidentiality of the data. Participation was voluntary and written informed consent was obtained from each participant prior to the start of the study. The participants were interviewed face-to-face to obtain information on sociodemographic characteristics, HBV vaccination, work-related and other risk behaviors. Blood specimens (10 mL) were collected from all participants, and serum samples were tested by enzyme-linked immunosorbent assay (ELISA) for the presence of HBsAg, total anti-HBc, anti-HBs, anti-HCV and anti-HIV using commercial kits (Biokit S. A., Barcelona, Spain). HBsAg-positive samples were assayed for HBeAg, anti-HBe and anti-HBc IgM by electroimmunoassay (ECLIA) using the Cobas® e601 Analyser (Roche Diagnostics, Mannheim, Germany); positive samples for anti-HIV were also confirmed by this method. Anti-HCV-positive samples were confirmed by “line immunoassay” (INNO-LIA III HCV Ab, Innogenetics, Belgic). HBV exposure was defined as a positive total anti-HBc and/or HBsAg test result. HBV vaccination like profile included all participants with the anti-HBs positive result ( $\geq 10$  mIU/mL) combined with negative total anti-HBc and HBsAg results. Participants lacking total anti-HBc, anti-HBs and HBsAg were considered serologically susceptible to HBV infection.

HBV DNA was extracted from HBsAg-positive and all anti-HBc-positive samples using the High Pure Viral Nucleic Acid kit (Roche Applied Science, German), strictly according to the manufacturer's instructions. The partial S genome region was amplified using a semi-nested polymerase chain reaction (PCR). Nucleotide

sequences of pre S/S/Pol HBV genes were determined by direct sequencing using BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA). Sequencing reactions were analyzed on an ABI 3730 automated sequencer (Applied Biosystems). Multiple sequence alignment was performed by using Clustal X program with HBV consensus sequences from all HBV known genotypes. Phylogenetic analysis was performed using the neighbor-joining method (bootstrap resampling test with 1000 replicates) in MEGA version 7 software.

The prevalence rate of HBV exposure and HBV vaccination like profile was calculated with a 95% confidence interval (95% CI). Chi-square and Fisher's exact test were initially used to determine the relationship between HBV exposure or HBV vaccination like profile and each independent variable estimating the odds ratio (OR) in univariate analysis. The variables with a *p*-value of 0.20 or less were included in multivariate Poisson regression models and a *p*-value of 0.05 or less was considered significant. All analyses were performed using the SPSS v. 22 for Windows, SPSS Inc., Chicago IL, USA. Package v 11. Additionally, Ives-Gibbons coefficient was used to assess the degree of agreement between self-reported HBV vaccination and serological HBV vaccination like profile (Ives and Gibbons, 1967). Ives-Gibbons coefficient was interpreted as follows: (a) null: when  $-1.0$ ; (b) insignificant agreement: between  $-0.99$  and  $-0.50$ ; (c) fair agreement: between  $-0.49$  and  $0.00$ ; (d) moderate agreement: between  $0.01$  and  $0.40$ ; (e) substantial agreement: between  $0.41$  and  $0.60$ ; (f) almost perfect agreement: between  $0.61$  and  $0.99$ ; and (g) perfect agreement: when  $+1.00$ .

The team doctor provided primary care to the participants and, when necessary, referred them for treatment or vaccination at the University Hospital of the Federal University of Mato Grosso do Sul. In addition, all participants received information about preventing sexually transmitted infections. The study protocol was approved by the Ethics Committee of the Federal University of Mato Grosso do Sul (CAAE 46914815.0.0000.021/15).

## Results

Approximately 330 recyclable waste collectors were contacted and 278 consented to participate in the study. The individuals were 18–70 years old (median age: 33 years); 50.4% were female and 60.1% worked at the dumping ground. The majority of the participants had a regular sexual partner (72.7%) and reported a monthly income of \$ 376 USD or less (56.8%). Of the total, 40.3% had worked at least 10 years as a recyclable waste collector (the median was 6 years (Inter Quartile Range (IQR): 2–12) and 33.8% reported previous waste sharps/needles accidents. In addition, 24.8% related a history of previous incarceration, 25.9% were illicit drug users, and 37.8% consumed alcohol more than three times a week.

The prevalence rate of HBV exposure was 10.1% (95% CI: 6.5–13.6). Only 0.4% was characterized as an HBV chronic carrier (HBsAg/total anti-HBc positive and anti-HBc IgM negative), 7.9% had been infected with HBV and had developed natural immunity (total anti-HBc/anti-HBs positive), and 1.8% were positive only for total anti-HBc. The majority of the participants (63.7%; 95% CI: 58.0–69.3) was considered susceptible to HBV infection (had no HBV serological markers) and only 26.3% (95% CI: 21.1–31.4) had a serological HBV vaccination like profile (Table 1 - Prevalence of hepatitis B virus serological markers among 278 recyclable waste collectors in Central Brazil, 2014–2016). Among participants with a serological HBV vaccination like profile (73/278), the majority were women (60.3%) and had 9 years or more of study (56.2%).

The HBsAg-positive sample also presented HBeAg-positive/anti-HBc IgM negative status. HBV DNA was successfully amplified and the sample was classified as genotype A. Occult hepatitis B infection (OBI) was not found among 27 HBsAg negative/anti-HBc positive samples.

**Table 1**

Prevalence of hepatitis B virus serological markers among 278 recyclable waste collectors in Central Brazil, 2014–2016.

Markers	N	%	95% CI <sup>a</sup>
Exposed			
HBsAg/anti-HBc	01	0.4	0.1–0.6
Anti-HBc only	05	1.8	1.3–2.3
Total anti-HBc/anti-HBs	22	7.9	4.7–11.1
Any HBV infection marker	28	10.1	6.5–13.6
HBV vaccination like profile			
Anti-HBs only	73	26.3	21.1–31.4
Absence of marker, considered susceptible	177	63.7	58.0–69.3

<sup>a</sup> Confidence Interval.

Anti-HCV was found in two (0.7%) participants who also had HBV past infection. Anti-HIV was found in three (1.1%) participants, two of them already knew they were infected.

In univariate analysis, the seroprevalence of HBV exposure was higher among recyclable waste collectors who were  $\geq 45$  years old (Figure 1), considered illiterate (Figure 2), injecting drug users (IDU), and those who reported previous homosexual contact and that had exchanged sex for money. Age  $\geq 45$  years (OR = 7.15;  $p = 0.001$ ), history of homosexual contact (OR = 5.29;  $p = 0.022$ ), tattoo (OR = 4.92;  $p = 0.009$ ) and surgery (OR = 2.89;  $p = 0.035$ ) were independently associated with HBV exposure (Table 2 - Factors associated with hepatitis B virus exposure among recyclable waste collectors in Central Brazil, 2014–2016).

Age 18–25 years (OR = 4.63;  $p = 0.000$ ), educational level  $\geq 9$  years (OR = 1.98;  $p = 0.029$ ) and knowledge of HBV transmission (OR = 3.08;  $p = 0.003$ ) were associated with serological HBV vaccination like profile (Table 3 - Factors associated with serological HBV vaccination like profile (isolated anti-HBs  $\geq 10$  mIU/mL) among recyclable waste collectors in Central Brazil, 2014–2016). Interestingly, 45.0% of the studied population self-reported at least one dose of HBV vaccination. Among them, only 34.4% had an HBV vaccination like profile and 60.8% had no HBV serological markers. In addition, among recyclable waste collectors who reported 3 HBV vaccine doses ( $n = 40$ ), only 45.0% presented the HBV vaccination like profile (Figure 3). The remaining 55% of them, who were actually considered susceptible to HBV infection, would not get vaccinated during any vaccination campaigns because they believed they were previously immunized. According to the coefficient of association of Ives and Gibbons, the degree of agreement found between the self-reported

HBV vaccination status and isolate anti-HBs positivity ( $r_{204} = 0.14$ ) was considered moderate.

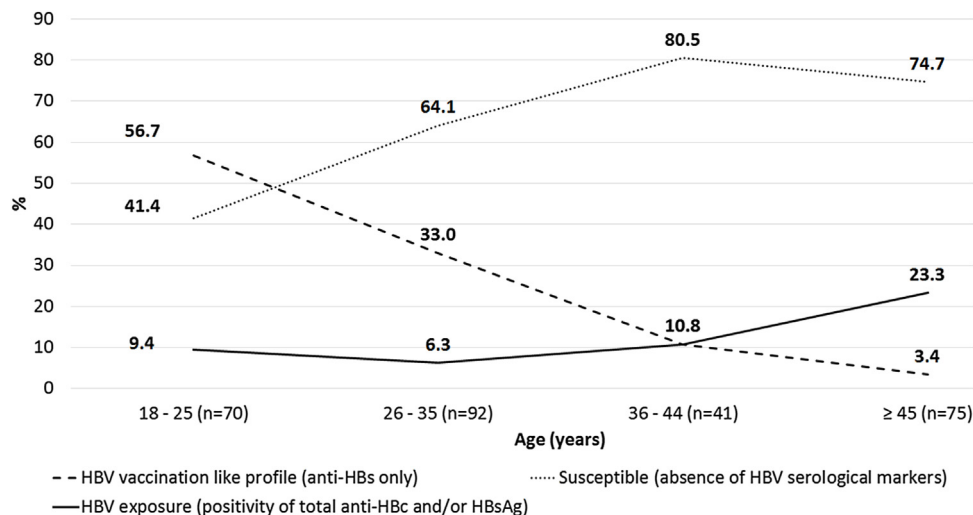
## Discussion

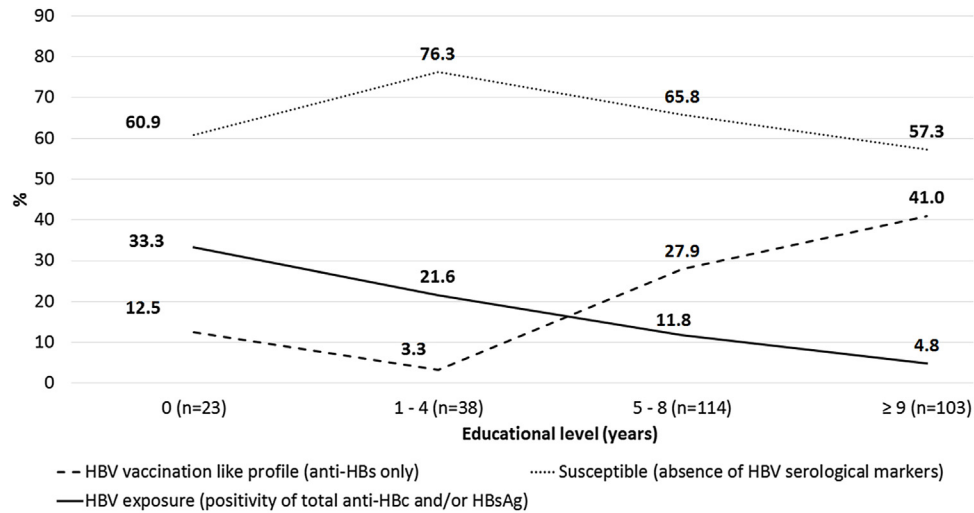
To our knowledge, this is the first HBV epidemiological study involving recyclable waste collectors recruited from both dumping ground and recycling cooperatives. Although this activity was regulated by the Brazilian Occupational Classification in 2002, individuals included in this study are still characterized by poverty, low education, and poor living conditions of life, housing and health. As observed in other studies involving recyclable waste collectors, the consistently higher risks of acquiring hepatitis infection are alarming (Rozman et al., 2008; Marinho et al., 2014).

In this study, a high overall prevalence rate of HBV exposure (10.1%; 95% CI: 6.5–13.6) was found, which was 3.3-fold higher than that reported in first-time blood donors in the same region (3.04%; 95% CI 2.7–3.4) (Lindenberg et al., 2013). Among recyclable waste collectors, this prevalence rate of HBV exposure was similar to that described in Goiânia, central Brazil (12.8%; 95% CI: 9.8–16.2) (Marinho et al., 2014) and lower than that observed in Santos, southeast Brazil (34.4%; 95% CI: 28.5–40.2) (Rozman et al., 2008), which had different methodology and sampling including informal recyclable waste collectors.

The low rate of HBV chronic infection found in this study (0.4%) and high rate of HBV exposure suggest that the infection occurred in adulthood. This hypothesis was confirmed by statistical analysis, which revealed that age  $\geq 45$  years and a history of tattoo, surgery and homosexual contact were associated with serological evidence of past or present HBV infection among recyclable waste collectors.

The association between HBV exposure and older age has been commonly reported and suggests that people born before vaccination programs were more exposed to the risk of infection and, over time, there is an increase and cumulative risk for HBV infection linked to percutaneous and sexual exposures (Tavares-Neto et al., 2004; Matos et al., 2008; Marinho et al., 2014; Villar et al., 2015). In addition, history of surgery and tattoos also remained associated with the risk of HBV exposure in this study, which reinforces the blood pathway as an important route for HBV infection (Cocchi et al., 1984). On the other hand, HBV exposure decreased among participants with higher educational levels, which might be due to awareness of HBV prevention, including the safe handling of recyclable waste and HBV vaccination (Figure 2).

**Figure 1.** HBV serological profile among recyclable waste collectors ( $n = 278$ ) according to age (years) in Central Brazil, 2014–2016.



**Figure 2.** HBV serological profile among recyclable waste collectors (n=278) according to educational level (years) in Central Brazil, 2014–2016.

**Table 2**

Factors associated with hepatitis B virus exposure among recyclable waste collectors in Central Brazil, 2014–2016.

Variables	HBV exposure no. <sup>a</sup> (%)	OR (95% CI)	p-Value	Adjusted OR (95% CI)	p-Value
<b>Age</b>					
18–44	11/132	8.3	1	1	
≥45	17/73	23.3	3.34 (1.47–7.60)	7.15 (2.23–22.92)	0.003*
<b>Education (years)</b>					
1–16	21/184	21.6	1		
Illiterate	7/21	33.3	3.88 (1.41–10.71)		0.006*
<b>Workplace</b>					
Cooperative	7/74	9.5	1		
Dumping ground	21/131	16.9	1.70 (0.76–3.80)		0.188
<b>Condom use<sup>b</sup></b>					
Always	4/53	7.5	1		
Sometimes/never	24/151	15.9	2.11 (0.77–5.79)		0.166
<b>Other risk behavior</b>					
History of Surgery	20/115	17.4	2.16 (0.90–5.16)	2.89 (1.08–7.74)	0.035*
History of Blood Transfusion	5/26	19.2	1.62 (0.55–4.70)		0.376
History of Tattoos	13/73	17.8	1.69 (0.76–3.78)	4.92 (1.49–16.21)	0.009*
Shared personal sharp objects	17/91	18.7	2.15 (0.95–4.86)		0.061
Illicit drugs	10/52	19.2	1.79 (0.77–4.17)		0.176
IDU <sup>b</sup>	2/3	66.7	13.46 (1.18–153.76)		0.050*
History of STI <sup>b</sup>	6/26	23.1	2.13 (0.77–5.87)		0.138
Exchanged sex for money	7/19	36.8	4.58 (1.63–12.93)		0.002*
Previously had homosexual contact	5/12	41.7	5.28 (1.55–18.02)	5.29 (1.27 – 22.15)	0.022*

95% CI: 95% confidence interval; OR: Odds ratio; IDU: intravenous drug user; STI: sexually transmitted infections. <sup>a</sup>Individuals with anti-HBs alone were excluded, since it probably indicates a previous vaccination (n = 205). <sup>b</sup>The total represents the number of individuals who answered the question. <sup>c</sup>Adjusted for age, education, work place, condom use, history of surgery, tattoos, shared sharp objects, illicit drugs, IDU, history of STI, exchange sex for money and homosexual intercourse.

In this investigation, those who reported previous homosexual contact were 5.3 times more likely to be infected. All of them were men, suggesting that mucosal lesions caused by anal sex may have been associated with transmission of several sexually transmitted

infections (STIs) (Jansen et al., 2015). In addition, most of them related illicit drug use, irregular condom use, exchanging sex for money, history of incarceration and STIs. These results suggest unprotected anal intercourse associated with behavioral factors for

**Table 3**Factors associated with serological HBV vaccination like profile (isolated anti-HBs  $\geq 10$  mIU/mL) among recyclable waste collectors in Central Brazil, 2014–2016.

Variables	HBV vaccination like profile no. <sup>a</sup> (%)	OR (95% CI)	p-Value	Adjusted OR (95% CI)	p-Value
Gender					
Male	29/122	23.8	1		
Female	44/128	34.4	1.68 (0.97–2.92)	0.065	
Age					
$\geq 26$	35/183	19.1	1	1	
18–25	38/67	56.7	5.54 (3.02–10.17)	4.63 (2.39–8.98)	0.000*
Education (years)					
1–8	32/150	21.3	1	1	
$\geq 9$	41/100	41.0	2.56 (1.47–4.48)	1.98 (1.07–3.65)	0.029*
Workplace					
Dumping ground	36/146	24.7	1		
Cooperative	37/104	35.6	1.69 (0.97–2.93)		0.061
Condom use <sup>b</sup>					
Always	15/64	23.4	1		
Sometimes/never	56/182	30.8	0.69 (0.36–1.33)		0.266
Tatoos	41/101	40.6	2.50 (1.43–4.36)		0.001*
History of STI <sup>b</sup>	5/25	20.0	0.58 (0.21–1.62)		0.294
Knowledge of HBV transmission <sup>b</sup>	22/49	44.9	2.40 (1.26–4.57)	3.08 (1.48–6.42)	0.003*

95% CI: 95% confidence interval; OR: Odds ratio; STI: sexually transmitted infections. <sup>a</sup>Individuals with anti-HBc positive were excluded (n = 250). <sup>b</sup>The total represents the number of individuals who answered the question. <sup>c</sup>Adjusted for gender, age, education, work place, tattoos and knowledge of HBV transmission.

hepatitis B infection (Seage et al., 1997; Remis et al., 2000; Neaigus et al., 2007; Nunes et al., 2007; Rozman et al., 2008).

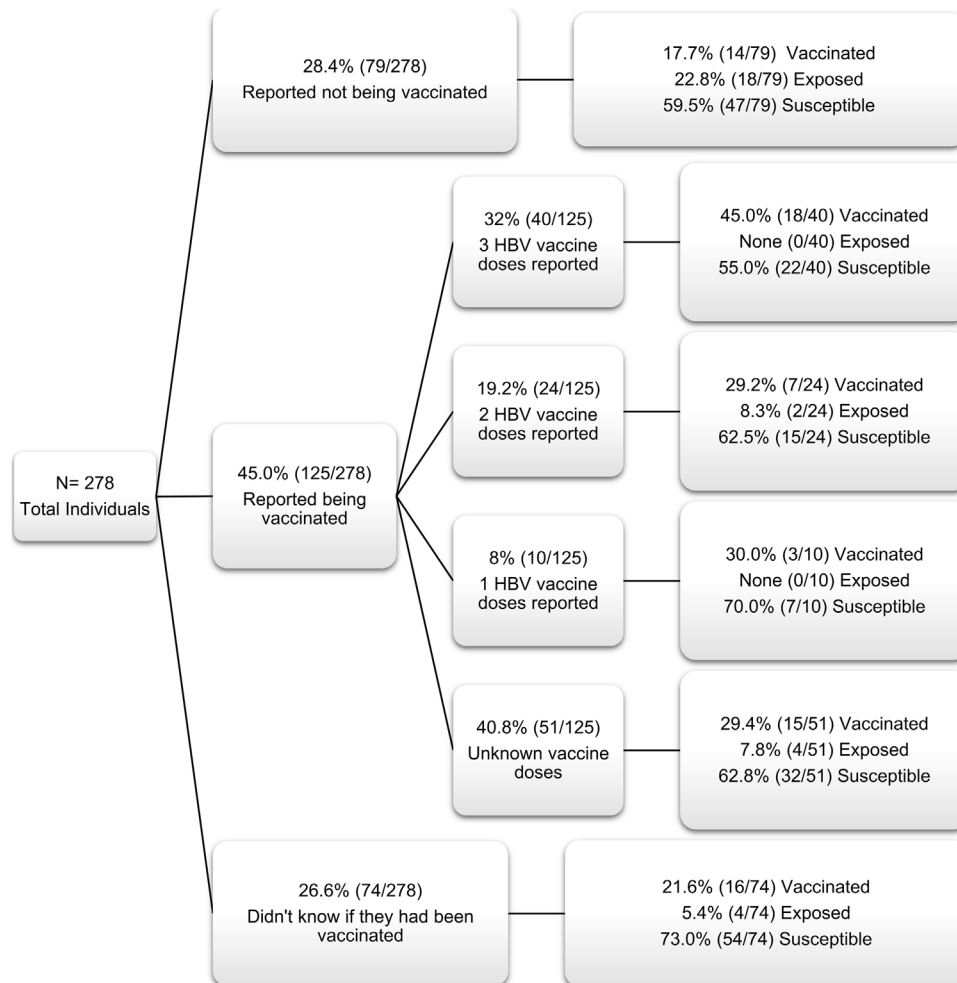
A low prevalence of chronic hepatitis B (0.4%; 95% CI: 0.1–0.6) was found in this study. The HBsAg-HBV DNA-positive subject was a young woman (22 years old), chronically infected, probably by vertical transmission. This hypothesis is supported by the fact that her mother, who also participated in the study, presented serological markers of past infection (total anti-HBc and anti-HBs). It is well known that around 90% of infants infected perinatally become chronic carriers (Edmunds et al., 1993; Motta-Castro et al., 2005; Ott et al., 2012). Moreover, OBI, investigated only in anti-HBc-positive samples, was not found. These results were expected since the prevalence of OBI varies, being low in regions of low endemicity (Torbenon and Thomas, 2002; Lindenberg et al., 2013; Makvandi, 2016).

Although HBV vaccine is available and is the most effective strategy to prevent HBV infection (Wang et al., 2018, 2019; Zhu et al., 2018), only 26.2% (95% CI: 21.1–31.4) of the studied population was positive for isolated anti-HBs over 10 mIU/mL (serological HBV vaccination like profile). Age under 26 years associated with the immune response to HBV vaccine was expected, since free hepatitis B vaccine has been provided to all newborns and to high-risk individuals since 1999 and gradually extended to older ages. But still, the majority 63.7% (95% CI: 58.0–69.3) of the studied population remains unvaccinated, especially those above age 45 years (Figure 1). This can be explained by the lack of regular education, with a resulting lack of knowledge about the importance of vaccines. The association between serological HBV vaccination like profile and knowledge of HBV transmission and the educational level  $\geq 9$  years reinforces these findings. In other words, low educational level and lack of knowledge about HBV transmission were factors associated with susceptibility

to HBV infection. Moreover, among those who reported being vaccinated with 3 doses, only 45% had a serological HBV vaccination like profile (Figure 3), suggesting that antibody levels declined over time (which we cannot prove since the participants did not have proof of vaccination and time) or self-reported hepatitis B vaccine have moderate sensitivity among this population. This finding may be explained by the fact that most of them did not understand or did not remember the type of vaccine that they have taken.

The high susceptibility to HBV infection of this population also may be justified by the fact that recyclable waste collectors are not classified as a population at high-risk by the Brazilian government, and only in 2015 was the hepatitis B vaccine released to the entire population, regardless of age and risk. In addition, most of the population studied faces the problem of not having permanent residency recognized by the city hall because they live in irregular areas, which makes it difficult to be seen in basic health units. In order to solve this problem, the team doctor provided primary care to the participants and referred all susceptible participants for vaccination at the University Hospital of the Federal University of Mato Grosso do Sul.

HCV infection was found among two participants with HBV exposure and  $\geq 45$  years old, who reported irregular condom use, tattoo, history of incarceration, and illicit drugs use. HBV and HCV are usually transmitted through the parenteral route, including exposure to shared needles or syringes and injury with infected sharps. Among the three HIV-infected, only one had serological HBV vaccination like profile; one was positive for total anti-HBc and anti-HBs and had a history of homosexual intercourse, drug use, and sex for money; and the last one was considered susceptible to HBV infection and reported having been arrested, drug use, and exchanging sex for money. Therefore, other STIs



**Figure 3.** The algorithm of self-reported HBV vaccination and HBV vaccination like profile among 278 recyclable waste collectors in Central Brazil, 2014–2016. Vaccinated: HBV vaccination like profile (isolated anti-HBs  $\geq 10$  mUI/mL); Exposed: positivity of total anti-HBc and/or HBsAg; Susceptible: absence of HBV serological markers.

should be investigated in this population. Many STIs, such as syphilis, HPV, chlamydia, and gonorrhoea, may increase the risk of exposure for HIV, HBV, and other pathogens (Galvin and Cohen, 2004; Mayer and Venkatesh, 2011).

This study has some limitations. The relationship between cause and effect cannot be determined. Social desirability bias might have led to over-reporting of preventive behavior once the findings were based on self-reports. In addition, participants' vaccination documents could not be accessed to verify vaccination data. Since some HBV vaccinated individuals lose detectable levels of anti-HBs over time, the frequency of susceptibility might be overestimated.

In conclusion, the identification of low rates of HBV vaccination like profile and a high level of HBV exposure in a low endemic region draw attention to the need for preventive measures, such as developing education, vaccination and screening strategies especially targeting more vulnerable populations. Because adult participants with a low educational level and lack of knowledge about HBV transmission are factors associated with susceptibility to HBV infection, vaccination campaigns using accessible language to the most disadvantaged populations can reduce the number of people susceptible to HBV infection.

### Conflicts of interest

The authors declare no conflict of interest.

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