



## **Seroprevalence and Associated Risk Factors of Hepatitis B Virus Infection in Bouaké, Côte D'Ivoire**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author AD designed the study, wrote the protocol, coordinated the research and wrote the first draft of the manuscript. Authors YLCK, SDA and RA helped with sample collection and laboratory analysis. Author SFT performed statistical analysis. Authors AMK, SK, JONT and PM helped with data collection and management. Authors EAA and AT helped with literature search. Author CAK was our mentor and consultant. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aim:** Determine the prevalence of serological markers and identify risk factors associated with Hepatitis B Virus (HBV) infection in patients screened at the Bouake teaching hospital.

**Study Design:** Retrospective cross sectional study was conducted

**Study Site and Period:** Bacteriology-Virology Laboratory/Bouake teaching hospital, Côte d'Ivoire, from April 2016 to January 2018.

**Methodology:** In all 1076 study participants, venous blood sample was collected and screened for HBV surface antigen (HBsAg) and antibody against HBV core antigen (anti-HBc), by electrochemical-luminescence following the manufacturer protocols. Additionally, questionnaires were used to collect information regarding sociodemographic variables and possible risk factors for hepatitis B infection. Data were processed and analyzed using EPI INFO 7 software.

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**Results:** A total of 1076 participants were included in this study with a median age of 30.0 years (range: 3 months; 82 years). Of which, 514 (48%) were female and 562 (52%) were male with female / male ratio 1.09. HBsAg was detected in 24,3% of participants and 82 1 (76,3%) were exposed to the risk of HBV infection (anti-HBc positive). High rate of HBV infection was detected in male (27.93%) ( $p=0.003$ ). The age group of 15–45 years were more infected (27.18%) ( $p<0.0001$ ). The detection rate of HBe antigen (HBeAg), anti-HBe and anti-HBc (total antibodies) were respectively 12%; 86% and 7%. Of 938 participants who were not vaccinated against HBV, 240 (25.58%) were HBsAg-positive. HBV vaccine uptake was protective against HBV infection (AOR =0.580; 95% CI 0.359-0.938;  $p=0.024$ ).

**Conclusion:** The rate of carriage of HBs antigen was higher than national rate, which confirms that Bouake is a highly endemic area for HBV infection. Vaccination against Hepatitis B virus is the only way to prevent and to fight effectively against this infection. It is therefore important to encourage the screening and vaccination in the general population.

*Keywords: Hepatitis B; HBsAg; anti-HBc; Bouake; Côte d'Ivoire.*

## 1. INTRODUCTION

Hepatitis B virus (HBV) infection is a major public health problem, with more than 360 million chronic carriers in the world [1,2]. About 500.000 to 1.2 million HBV-infected people die yearly from cirrhosis of the liver and hepatocellular carcinoma (HCC) worldwide [3,4]. HBV is the most common and the most contagious worldwide. In 2015, more than 1.34 million people died from HBV infection. This figure is higher than death from tuberculosis (TB) and the human immunodeficiency virus (HIV) infection. However, mortality attributable to TB and HIV is decreasing, while the one due to hepatitis is constantly increasing [5,6]. The HBV reservoir is constituted by the large number of chronic carriers worldwide [7]. Studies reported that factors such as unprotected sexual intercourse, needle stick injuries, splash, intravenous and/or intramuscular injections, blood transfusion, and unvaccinated population have been associated with increased risks of hepatitis B infections [8,9]. Vertical transmission is the main route of disease transmission to children from mothers, whereas main means of transmission for sexually active partners is sexual intercourse [9,10]. Côte d'Ivoire is a highly endemic area for HBV with a prevalence estimated at 8% - 12% in the general population [6]. A study conducted in Abidjan in 2019 detected a prevalence of HBsAg of 5.3% among students of secondary school [11]. Another study conducted in 2001 allowed to detect a 12.5% prevalence of HBsAg among blood donors in Bouaké [12]. The increasing prevalence of HBV in Côte d'Ivoire requires adequate management and the implementation of effective preventive measures to limit the spread of the virus. One of the main routes of HBV-transmission might be blood transfusion, especially when donated blood is not tested for

HBs antigen (HBsAg) [3,13]. Systematic screening for hepatitis B surface antigen in blood donors and in pregnant women is necessary to identify and promptly treat those infected [9]. Despite the scientific advances and opportunities for management and treatment of patients, most people infected with HBV remain unaware of their status and therefore frequently discovered with advanced disease and may have transmitted infection to others [6]. A Cross-Section Study conducted in Abidjan in 2011 on Vaccination Coverage against Hepatitis B revealed a lack of knowledge about hepatitis among the general population [14]. The updated World Health Organization (WHO) recommendations indicate that all suspected cases of hepatitis B must be confirmed with a laboratory diagnostic test before treatment. Implementation of the Global Health Sector Strategy would prevent 7.1 million deaths between 2015 and 2030 [15,6]. There is growing body of literature in Côte d'Ivoire on HBV infection. However, so far, there are limited information on the prevalence of HBsAg and its associated risk factors in Bouaké. Therefore, this study was conducted to determine the prevalence of serological markers and to identify risk factors associated with HBV infection in patients screened at the Bouaké teaching hospital (CHU of Bouaké).

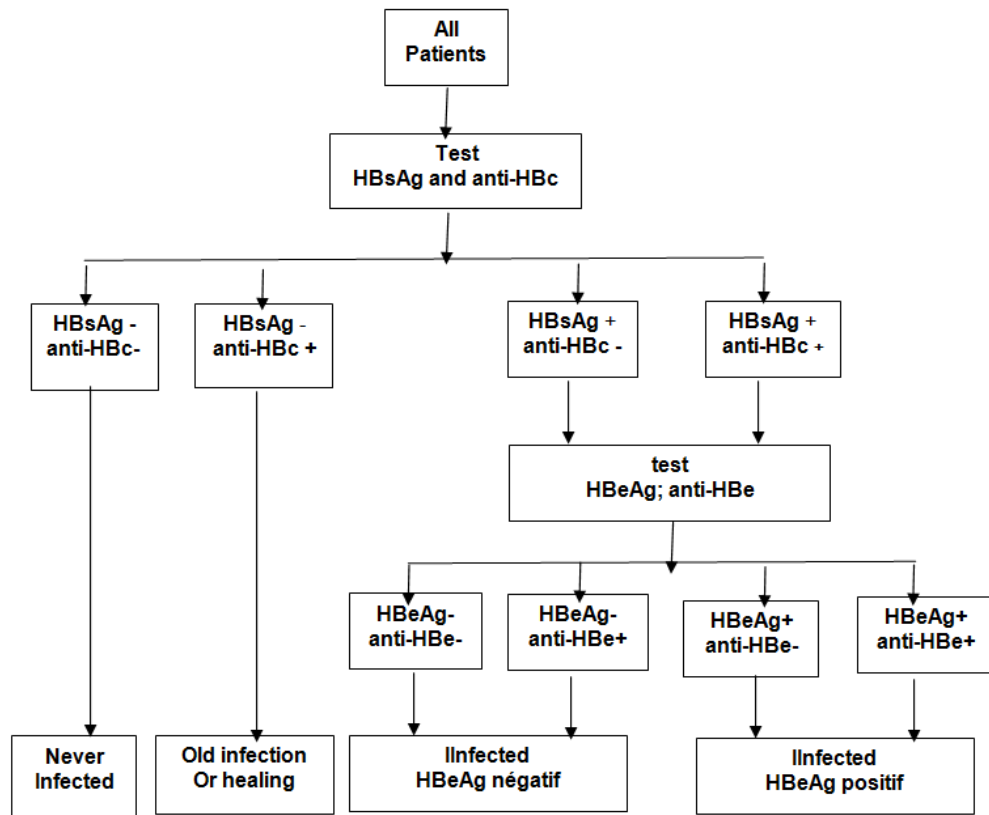
## 2. METHODOLOGY

### 2.1 Study Design and Period

This was a retrospective cross sectional study conducted from April 2016 to January 2018.

### 2.2 Study Site

The study was conducted at the Laboratory of CHU of Bouake, at the Microbial Serology Unit.



**Fig. 1. Flow diagram showing the method for hepatitis B serology testing. Anti-HBc, antibody to hepatitis B core antigen; anti-HBe, antibody to hepatitis B ‘e’ antigen; HBeAg, hepatitis B ‘e’ antigen; HBsAg, hepatitis B surface antigen**

Bouake is the second city of Côte d'Ivoire, located in the center at 350 km from Abidjan. The size of the city of Bouake is about 72 km<sup>2</sup> with an estimated population of 1,542,000 inhabitants. This population mainly includes indigenous "Baoulé ethnic group" and of Malinké and Burkinabe allogeneic people. The CHU of Bouake is built on an area of 23 hectares. It has currently a wide range of medical, surgical and biological specialties. The laboratory of CHU is composed by a microbiology unit (bacteriology-virology/parasitology-mycology), serology, biochemistry, immunology-hematology and two molecular biology platforms: one for the diagnosis of viral hepatitis and HIV and the other for the diagnosis of viral hemorrhagic fevers.

### 2.3 Study Population

The study population consisted of patients of all ages and both gender who attended the Laboratory of CHU of Bouake by their own or on medical advice for hepatitis B screening. A comprehensive sampling that took into account

all patient tests results correctly entered in the laboratory database during the study period.

### 2.4 Sample Collection

In all the study participants, about 3 milliliter (ml) of venous blood sample was collected by dry tube aseptically. After coagulation, the blood was centrifuged at 3000 rpm for 10 minutes. Sera was separated from each sample and the collected sera were immediately tested or stored in the refrigerator at -20°C for a maximum of 03 days. Samples were labeled with unique identification numbers which are identical to the code given in the questionnaire. All participants were screened according to the algorithm in Fig. 1 above. The sera of participants were analyzed for HBV surface antigen (HBsAg) and antibody to HBV core antigen (anti-HBc) by electrochemical-luminescence method, according to the manufacturer protocols. Samples reported to be positive for HBsAg were double checked for other markers of HBV infection (HBeAg, anti-HBe, anti-HBc type M).

## 2.5 Data Collection

Structured and pretested self-administered questionnaire was used to collect information regarding sociodemographic variables (age, sex, occupation, level of education) and possible risk factors for hepatitis B infection acquisition such as tattooing, blood transfusion history, vaccination status. The questionnaire was previously validated by the coordinating committee for viral hepatitis studies at Bouake teaching hospital.

## 2.6 Data Analysis

Data from the questionnaires and laboratory test results were entered into a database and analysed using EPI INFO 7 software. Descriptive statistics were used to describe the socio-demographic characteristics of the study participants. Khi-2 or Exact Fisher's test was used to compare the prevalence of HBV infection. The odds ratio (OR) was generated using bivariate binomial logistic regression model to determine risk factors associated with HBV infection among study participants. The significance threshold was set at 5%.

## 2.7 Ethical Consideration

The study was carried out with the approval of the Medical and Scientific Department (DMS) of CHU of Bouaké, which acts as the institutional ethics committee. Data confidentiality was preserved by assigning an anonymity number to each survey form. Prospective subjects were informed about the study and written consent

was obtained from those who agreed to participate. All questionnaires were stored in a secure room the information of study participants was secured in a password protected laptop.

## 3. RESULTS

### 3.1 Socio-Demographic Characteristics

A total of 1076 people were included in this study. The mean age of participants was 30 years. About 802 (74%) study participants were aged from 15 to 45 years while 175 (16%) were aged from 45 to 60 years. Over 562 (52.23%) participants were males. More than 499 (46%) study participants had a university education level and 149 (13%) participants had no education level. Thirty percent of study participants were pupils and students, and the public services workers accounted for 27% of the participants (Table 1).

### 3.2 Prevalence of HBV Serological Markers

Of the 1076 participants in this study, the prevalence of HBV infection (HBsAg) was 24.25% (261/1076) (Table 2). There was a significant difference in the prevalence of HBsAg between male (27.93%) and female (20.23%) ( $p=0.003$ ) in participants. The HBV infection rate gradually increased from 9.61% in those less than 15 years old to a peak of 27.18% in the age group of 15–45 years, and then it declined to 13.04% in the group of participants older than 60 years. The differences observed between age groups were significant ( $p=0.001$ ).

**Table 1. Distribution of socio-demographic data of the study population according to the variables studied**

Variables		Participants (N=1076)	Frequencies (%)
Sex	Female	514	47,77
	Male	562	52,23
Age (years)	Less than 15	52	4,83
	15-45	802	74,53
	46-60	175	16,26
	More than 60	46	4,27
Occupations	Pupils / students	331	30,76
	public servants	294	27,32
	Cultivators	72	6,69
	Housewives	99	9,2
	Informal sector	258	23,98
	Unemployed	22	2,05
	Primary	138	12,83
Education level	Secondary	290	26,95
	university	499	46,38
	Others	149	13,85

**Table 2. Serological markers to detect HBV infection**

Variables		Participants	Ag HBs + Nbre (%)	P. Value	Anti-HBCT Nbre (%)	P. Value
Sex	Female	514	104 (20,23)	0,003	460 (70,23)	< 0,0001
	Male	562	157 (27,93)		361 (81,85)	
Age (years)	Total	1076	261	0,001	821	< 0,0001
	Less than 15	52	5 (9,61)		13 (0,25)	
	15-45	802	218 (27,18)		615 (76,68)	
	46-60	175	32 (18,28)		151 (86,28)	
	More than 60	46	6 (13,04)		42 (91,30)	
Occupations	Pupils /students	331	68 (20,54)	0,081	224 (67,67)	< 0,0001
	public servants	294	75 (25,51)		241 (81,97)	
	Cultivators	72	26 (36,11)		65 (90,27)	
	Housewives	99	23 (23,23)		76 (76,76)	
	Informal sector	258	64 (24,80)		201 (77,90)	
	Unemployed	22	5 (22,72)		14 (63,63)	
	Primary	138	35 (25,36)		98 (71,01)	
Education level	Secondary	290	67 (23,10)	0,893	216 (74,48)	
	university	499	120 (24,04)		373 (74,74)	
	Others	149	39 (26,17)		134 (89,93)	

The infection rate was highest among farmers (36.11%), followed by public services workers (25.51%). The detection rate of HBsAg was even lower among unemployed participants ( $p=0.081$ ). Also, among patients with no education level, the infection rate was higher, followed by patients with primary, secondary and higher education level ( $p=0.893$ ).

Regarding the total anti-HBc, a marker of HBV exposure, the positivity rate was 76.30% in participants and a significant variation was noted between genders ( $p < 0.0001$ ).

HBV exposure increased with age in both sexes, and it reached a peak in the group older than 60 years (91.30%), indicating that exposure to HBV had occurred in every age group. The detection rate of anti-HBc was higher among farmers (90.27%), followed by salaried civil servants (81.97%), workers in the informal sector (77.90%) and housewives (76.76%) ( $p < 0.0001$ ). The positivity of anti-HBc was higher among study participants with no education ( $p < 0.0003$ ) (Table 2).

A total of 107 (40.99%) patients who were HBsAg-positive had sufficient sample to allow further testing for HBeAg, anti-HBe and anti-HBc IgM-type. A total of 103 (37%) were HBeAg-positive and 127 (45%) were anti-HBe-positive. The positivity rates were respectively 3.73% for patients in the acute phase of infection (anti-HBc IgM-type); 12.14% for patients in the active

replication phase of the virus (HBeAg); and 94.39.6% for those in the healing phase (anti-HBe Antibodies).

### 3.3 Factors Associated with HBV Infection

The following associated risk factors were assessed for hepatitis B virus infection: history of blood transfusion, history of vaccination status, tattooing, and blood exposure. Of the total study participants only 138 (12.82%) were vaccinated for HBV. Among those who were not vaccinated, 240 (25,58%) were positive for HBsAg and this was statistically significant (AOR = 0.58, 95% CI = 0.35–0.93,  $P = 0.0024$ ). Of 66 (6.13%) participants who had history of blood transfusion, 15 (22,72%) were positive for HBsAg (OR = 1.07, 95% CI, 0.60–1.91,  $P = 0.81$ ). Other factors such as tattooing, and blood exposure were not significantly associated with HBV infection (Table 3).

## 4. DISCUSSION

This study was carried out to determine seroprevalence of serological markers of HBV infection and risk factors associated with seropositivity among people tested in CHU of Bouaké. Though HBV infection is preventable and there is a protective vaccine, it remains a public health problem in Africa especially in Côte d'Ivoire.

**Table 3. Binomial logistic regression analyses for potential risk factors of HBV infection**

variables	Participants	AgHBS+ (%)	OR	95% CI for OR	P-value
<b>Blood transfusion</b>					
Yes	66	15 (22.72)	1.07	0.59 — 2.06	0.88
No	1010	246 (24.35)	1		
<b>Vaccination</b>					
Yes	138	21 (15.21)	1.68	1.03 — 0.286	0.03 *
No	938	240 (25.58)	1		
<b>Tattoo</b>					
Yes	6	0 (0)	-	0.28 — -1.00	0.60
No	1070	261 (100.00)	1		
<b>Blood exposure accident</b>					
Yes	20	6 (0.30)	0.804	0.31 — 2.54	0.62
No	1056	255 (24.14)	1		

\*Statistical significant less than 0.05

Serological testing revealed a global prevalence of HBsAg positivity of 24,25%. These results shows that Bouaké is a highly endemic area, with HBsAg carriage rate higher than the national prevalence of 10% [16,17]. The positivity rate of AgHBs was higher among male (27.93%) than female (20.23%) ( $p=0.003$ ). The study also showed that the detection rate of anti-HBc (marker of exposure to HBV infection) was 76,30% and HBeAg positivity rate (marker showing active replication of the virus) was 12,14%. These results, especially the high prevalence of HBsAg and current HBV infection, suggest that hepatitis B is a very serious health concern in Bouaké (Côte d'Ivoire).

In this study, HBV vaccine uptake were the only factors associated with HBV infection.

This high HBsAg positivity could be explained by the lack of implementation of the guidelines, screening and care algorithms suggested by the National Program for the control of Viral Hepatitis (PNLHV) and the Ivorian Network for the control of Viral Hepatitis (RILHVi) created in 2004 [14].

Indeed, the PNLHV is encountering structural difficulties due to the low budget al. located by the State and the lack of commitment from national and international donors. Because this pathology is not financially covered, the PNLHV does not have the resources needed to organize screening and awareness campaigns of the population [14].

This prevalence of HBsAg positivity obtained in our study is higher than that reported by Kra et al., 12.5% among blood donors in Bouaké [12] and that obtained by Ouattara A et al. among students in Abidjan which was 5.3% [11]. These

differences in reported prevalence might be explained by a difference in study methodology (study population and sample size). Other studies of HBsAg conducted by Balogun et al. in Nigeria among subjects infected with human immunodeficiency virus (HIV) reported a similar prevalence of 28.4% [18]. Alao et al. also reported a prevalence of 20% among potential blood donors in Otukpo, Nigeria [19]. In Cameroon, Noubiap and *coll.* reported a prevalence of 23.7% among HIV-infected individuals [20]. Furthermore, in the study implemented by Kabinda et al. in Congo, lower prevalence of 4.2% were reported among 1079 volunteer blood donors tested [21]. Then, a prevalence of 10.87% was found among 230 people voluntarily screened in Togo [22].

The study of the prevalence of HBsAg according to sex and age showed a predominance of infection in men with 27.98% versus 20.23% in women ( $p<0.05$ ). This could be related in one hand to the direct effect of the predominance of the male sex in the sample, and on the other hand to the fact that multi-sexual partnership is more frequent in men than in women. These results were similar to those reported by Kabinda et al. in DRC among voluntary blood donors [23] and those found by Bivigou-Mboumba B et al. in Gabon on viral hepatitis carriage [24]. The age of HBV infection acquisition is one of the determining factors in incidence and prevalence rates. In resource limited settings like Côte d'Ivoire, most HBV infection take place during childhood due to high endemicity nature of hepatitis B virus infection [8]. However, this was not supported by our study, which showed a significant association between age and HBsAg positivity ( $p<0.05$ ). More than 27.18% of HBsAg positive patients were between 15 and 45 years

of age. HBsAg carriage was more common in adults than children, which means many study participants, has been infected during adulthood. In a similar study of HBsAg carriage which was done in Mali by Dao et al., adults were more infected [25].

The majority of patients in this age group were public services workers and students. They constitute a very sexually active population, which would represent a high risk due to the sexual transmission of the virus [23]. One of the main routes of HBV transmission in our study would probably be the sexual route, hence the need to strengthen awareness, education and communication campaigns to control HBV and other sexually transmitted diseases. Other factors such as homosexuality and intravenous drug use have been documented in previous studies as factors associated with HBV infection [8]. In this study, only 5 (9.61%) HBsAg-positive patients were aged between 0 and 5 years, which indicated that the majority of patients were infected in adulthood. This low prevalence of HBV in children is interesting, as children are easily exposed to the disease, particularly through vertical transmission by their mothers. The low HBsAg carriage in children could also be explained by the introduction of hepatitis B vaccine in the expanded program of vaccination (PEV) in Côte d'Ivoire since 2002 [26]. The positive impact on the reduction of HBsAg carriage in children show the effectiveness of the HBV vaccine within the PEV in Côte d'Ivoire. Moreover, this low rate might also be the consequence of the low representation of children in our sample. Previous reports showed that more than 90% of exposed newborns and about 30% of children under 5 years of age fail to clear HBV from their body, which later progress to a chronic infection. However, 95% of infections acquired in adulthood result in spontaneous recovery [9]. In a Tanzania study conducted by Elichilla et al., HBV infection among health care workers was not associated with gender, and patients were infected mostly during childhood [8].

The results of our study showed a detection rate of HBsAg of 26.17% in people with no education. However, HBsAg positivity was not associated with education level. Other authors have reported that the prevalence of HBV was significantly higher among illiterate women than among those with high education level [27].

Regarding markers of exposure to HBV infection (total anti-HBc), 76.30% of the study participants

had already been in contact with HBV. The positivity of total anti-HBc was significantly associated with gender and age ( $p < 0.05$ ). Positivity was also associated with occupation and education level ( $p < 0.05$ ). In similar study conducted in Ethiopia, authors obtained a prevalence of total anti-HBc of 25.8% among 5009 pregnant women tested [27], while Yu-Ling *and coll.* reported a positivity of 13.3% in Sierra Leone study [15].

Out of 107 patients tested for follow-up markers of HBV infection, the positivity rate of HBeAg was 12.14%, and the positivity rate was 94% for anti-HBe. These rates were higher than those of Nahom *and coll.*, who reported a prevalence of 3.9% for HBeAg and 17.4% for anti-HBe [27].

Our results revealed that only 12.82% of the study population had a history of HBV vaccination. This low vaccination rate could be explained by a lack of information and a lack of knowledge of the disease in the general population. Indeed, the proportion of students aware of the existence of HBV is low, as shown by a study carried out in Abidjan in a secondary school [11]. Good level of knowledge of the disease was protective towards hepatitis B infection, therefore those with poor knowledge has more chances of being infected. Risk factor analysis showed that vaccination status was significantly associated with HBV infection as a protective factor (OR= 1.68; CI 1.03 – 2.86;  $p = 0.03$ ).

In this study more than two thirds of the participants were not vaccinated against HBV and of these 25.58% were HBsAg carriers. Other factors such as ethnicity, region of origin and religion might influence the odd of participants to be infected. However, in the current study these data could not be collected. The low purchasing power of the populations is an obstacle to the management of this affection and its complications. Given the inaccessibility of treatment due to the high cost, vaccination remains the only way to fight effectively against this disease.

However, among patients vaccinated against HBV, 21 (15.21%) were HBsAg positive. This could be explained by the fact that these patients were already exposed to HBV infection before vaccination or that they did not receive the three protective doses of the vaccine. In another study, authors showed that the percentage of good knowledge of HBV was very low and about half of the participants didn't know that antibody titer

should be done after finishing the third dose [8]. Other factors studied such as blood transfusion, tattooing, blood exposure accidents were not statistically associated with HBV infection. However, the results showed that 15 (22,72%) HBsAg-positive patients had a history of transfusion. In a similar Tanzania study, the authors showed that people with a history of blood transfusion were 21 times more likely to be infected with HBV than those who did not (OR=21.44; 95% CI; 6.05-76.01) [8]. To ensure transfusion safety in Côte d'Ivoire, the National Blood Transfusion Centre (CNTS) has been practicing systematic HIV screening since 1992, HBsAg (HBsAg) testing since 1993 and anti-HCV antibody testing since 1997. However, it has been reported that HBsAg-negative donors who were newly infected with HBV were capable of transmitting the virus. This risk is associated with donations collected during the virological and serological windows that precede the appearance of biological markers of infection, or during the pre-conversion phase of recent infection. In the later situation, HBsAg levels in the circulation is below detectable limits. The use of molecular tests or more sensitive serological tests to detect HBsAg might further reduce the risk of HBV transmission through blood transfusion [28].

## 5. CONCLUSION

The rate of carriage of HBs antigen was higher than national rate, which confirms that Bouake is a highly endemic area for HBV infection. More than three-quarters of study participants were exposed to potential risks of infection. Vaccination against Hepatitis B virus is the only way to prevent and to fight effectively against this infection. It is therefore important to encourage the screening and vaccination in the general population.

## CONSENT AND ETHICAL APPROVAL

The study was carried out with the approval of the Medical and Scientific Department (DMS) of CHU of Bouaké, which acts as the institutional ethics committee. Data confidentiality was preserved by assigning an anonymity number to each survey form. Prospective subjects were informed about the study and written consent was obtained from those who agreed to participate. All questionnaires were stored in a secure room, the information of study participants was secured in a password protected laptop.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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