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Analysis of hepatitis B Virus Test results among blood donors in Chongqing, China

Lan Wei^{1,2}, Min Chen¹, Fang Wang¹, Meijun Li¹, Dong Liu¹, Chengbing Xie¹, Dongyan Yang^{1*}, Siyang Wen^{3*} and Yongzhu Xu^{1*}

Abstract

Background Hepatitis B virus (HBV) infection is a major concern regarding blood safety in countries with a high HBV prevalence, such as China. We aimed to understand the prevalence of HBV infection among blood donors in Chongqing and provide an important basis for developing appropriate blood screening strategies.

Methods Dual enzyme-linked immunosorbent assays (ELISAs) for hepatitis B surface antigen (HBsAg) were conducted in parallel with nucleic acid testing (NAT) of donors. All HBsAg-reactive and/or HBV DNA-positive blood samples were tested for HBsAg and hepatitis B DNA levels.

Results A total of 117,927 blood donor samples were collected from the Chongqing Blood Center between April 2020 and November 2020. In total, 473 HBV-ineligible samples were retained for HBsAg and DNA confirmation. A total of 272 samples were confirmed to be HBsAg+, including 2 HBV DNA – and 270 HBV DNA + samples. A total of 201 donations were HBsAg–, including 72 HBV DNA – samples. The rate of HBV infection was 65.33% (309/473) in men, which was significantly higher than that in women ($p < 0.001$). The HBV failure rate was higher among the first-time donors ($p < 0.05$). Of the 182 NAT R/HBsAg N/N samples (Nucleic acid test reactivity/2 anti-HBsAg tests negative), 37.91% (69/182) were false positives. The proportion of hepatitis B infections in the 18 NAT R/HBsAg N/R (Nucleic acid test reactivity/1 anti-HBsAg tests negative) samples was 94.44% (17/18), of which 50% (9/18) were occult HBV infection. A total of 95.83% (69/72) of the false positives were from the NAT R/HBsAg N/N group, and 58.33% (42/72) were first-time donors.

Conclusion Our data showed a strikingly high HBV infection rate among blood donors in Chongqing. Double ELISA and single NAT can effectively prevent HBV leakage and improve blood safety. First-time donors have a high rate of HBV transplant failure; therefore, donors should be retained and recruited from low-risk groups.

Keywords Hepatitis B virus (HBV), Donors, Nucleic acid testing, Occult HBV infection, Screening strategies

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Background

Hepatitis B virus (HBV) infection is a global health threat, particularly in developing nations [1]. An estimated 257 million people worldwide are infected with chronic HBV infection [2]. Globally, 786,000 people die each year from liver diseases related to HBV [3]. Although China's compulsory hepatitis B vaccination policy was introduced in 1992, over 93 million people are HBV carriers, and 30 million have chronic HBV [4].

The transfusion risks associated with HBV transmission are recognized globally [5]. Transfusion of hepatitis B surface antigen (HBsAg)-negative blood with a low HBV DNA load has been shown to result in HBV infection [6]. In China, HBV is a mandatory screening test. Dual HBsAg enzyme-linked immunosorbent assays (ELISAs) has been adopted parallel to nucleic acid testing (NAT), which began in donors in 2015. NAT can be performed using either a single sample or a combination of multiple samples; however, discrepancies between HBsAg and HBV DNA results have been reported in 6–9% of blood donations [7]. It is necessary to study and analyze HBV screening results in this region because of the variations in reagent performance and screening strategies. This will assist in developing more efficient blood screening strategies and help to evaluate the detection performance of current reagents and strategies on a scientific basis.

Screening practices for HBV infection vary across countries in terms of reagent manufacturers and testing procedures. Over the past three decades, the risk of transfusion-transmitted HBV (TT-HBV) has reduced significantly with the introduction of screening for antibodies against the hepatitis B core antigen (anti-HBc) in some countries, the use of NAT, and improved donor recruitment procedures [8, 9]. In recent years, the Chinese government has taken several steps to improve blood supply safety, including promulgating a new blood donation law in 1998 and screening procedures in 2012, which are mandatory and guide how we evaluate donor eligibility, screen donors, and manage donations [10]. However, the risk of TT-HBV infection remains higher than that of other screened viruses, such as hepatitis C virus (HCV) and human immunodeficiency virus (HIV).

We analyzed the outcomes of 117,927 blood donations received from the Chongqing Blood Center between April 2020 and November 2020. This study aimed to evaluate the HBV blood-screening strategy in Chongqing and provide a scientific basis for donor recruitment, retention, and return.

Materials and methods

Subjects of the study

117,927 blood donor samples from Chongqing Blood Center were collected between April 2020 and November

2020. These samples were screened according to the relevant provisions of the Requirements for Health Examination of Blood Donors (GB18467-2011). Data was obtained from the Blood Station Information Management System (BMIS).

Instruments and reagents

The instruments included a spiking device (Xantus, Shenzhen Akcome), an enzyme immunoassay analyzer (FAME24/20, Hamilton, Switzerland), a nucleic acid detector (Tigris/Panther, Grifols, Spain), and a biochemistry analyzer (AU640/680, Beckman, USA), all of which were calibrated. Reagents include HBsAg (Beijing Wantai/Italy Sorin) and nucleic acid screening (NAT) reagents Ultrio Plus/Elite (Galliford, Spain). These reagents are qualified and used within the expiry date.

Primary screening

Finger stick blood was collected for primary screening of ALT, HBsAg, and anti-TP before donation. Samples were kept in side bags at the time of blood collection for ELISA and NAT. HBsAg was detected using two different reagents. Nucleic acid testing (TRI-NAT) was performed individually using the Grifols system (Tigris/Panther) and reactive samples were tested for HBV/HIV/HCV differentiation. Adjudication criteria: HBsAg test result $S/CO \geq 0.8$ is the critical value; if double reagent ≥ 0.8 , it is considered reactive; if single reagent $S/CO \geq 0.8$, retest with the same reagent in two wells and any well ≥ 0.8 , it is considered reactive; NAT test result $S/CO \geq 1.0$, it is considered reactive. All available HBsAg reactive and/or HBV DNA positive plasma samples were collected from discarded plasma bags and sent to the National Centre for Clinical Laboratories. Anonymized individual demographic information was collected from BMIS, including sex, age, ethnicity, occupation, and blood grouping.

Validation of HBsAg and HBV DNA

All HBsAg reactive and/or HBV DNA positive blood samples were further tested for HBsAg using the ARCHITECT HBsAg Qualitative II (Abbott, LOD: 0.05 IU/mL). Reactive samples with signal levels (S/CO) between 1.0 and 5.0 were retested using the Elecsys HBsAg II (Roche, LOD: 0.05 IU/mL), the Cobas TaqScreen MPX assay, version 2.0 (Roche, LOD: 2.3 IU/mL) and the HBV DNA/HCV RNA/HIV (1+2) RNA Diagnostic Kit (Livzon, LOD: 5 IU/mL) for nucleic acid identification. A summary of the HBsAg and HBV DNA assays is shown in Fig. 1.

Statistical analysis

SPSS 22.0 statistical software was used to analyze the data. Count data were expressed as cases or percentages. The X^2 test was used to compare the count data between

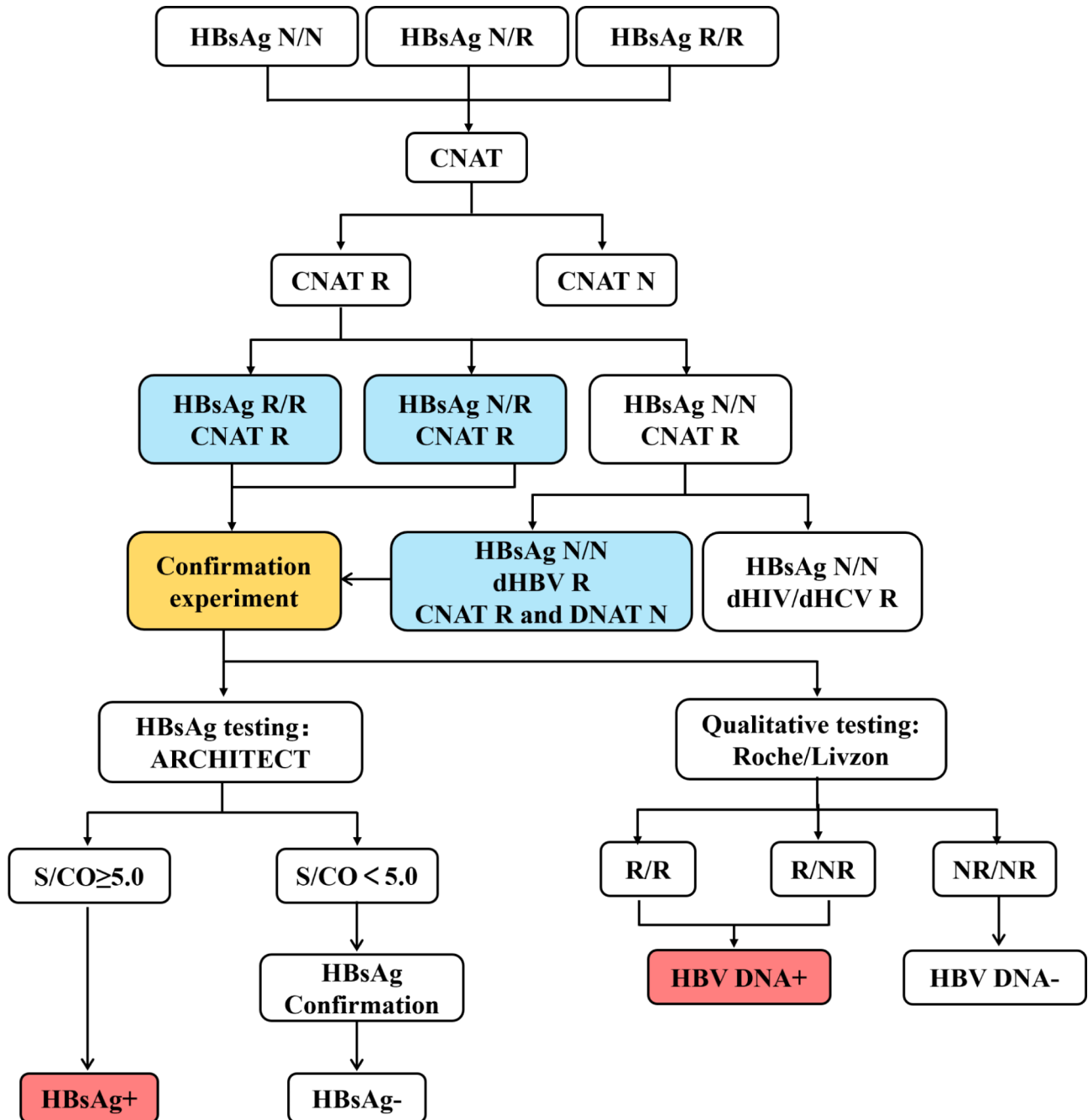


Fig. 1 Confirmatory process of HBsAg and HBV DNA. +: positive; -: negative; R: reactive; N: nonreactive; CNAT: HBV/HIV/HCV combined nucleic acid test; DNAT: HBV/HIV/HCV differential nucleic acid test. ARCHITECT HBsAg, ARCHITECT HBsAg Qualitative II (Abbott Ireland Diagnostics Division, Sligo, Ireland); HBsAg Confirmatory, ARCHITECT HBsAg Qualitative II Confirmatory (Abbott Ireland Diagnostics Division), or HBsAg Confirmatory Test (Roche Diagnostics GmbH). Roche, cobas TaqScreen MPX Text, version 2.0 (Roche Molecular Systems, Inc., Branchburg, NJ, United States); Livzon, HBV DNA/HCV RNA/HIV (1 + 2) RNA Diagnostic Kit (PCR Fluorescence Probing) (Livzon Diagnostics, Zhuhai, China)

the two groups. $P < 0.05$ indicated that the difference was statistically significant.

Results

Validation of HBsAg and HBV DNA

A total of 117,927 blood samples were collected between April and November 2020 (Fig. 2). In total, 473 HBsAg-reactive and/or HBV DNA-positive samples were included in this study. Finally, 272 samples were confirmed to be HBsAg+, including 2 HBV DNA–samples and 270 HBV DNA+samples. A total of 201 samples were HBsAg–, including 72 HBV DNA–.

Characteristics of enrolled donors

After screening for HBsAg and HBV DNA, 473 samples were subjected to confirmatory testing. The characteristics of the enrolled donors are summarized in Table 1. The HBsAg+, HBsAg–/HBV DNA+, and HBsAg–/HBV DNA–groups were further analyzed regarding the patients demographic information. The proportion of males was higher in the HBV-infected groups ($p < 0.001$), especially in the HBsAg–/HBV DNA+group, of which 71.32% (92/129) were male. Donors older than 46 years had higher rates of HBsAg+and HBV DNA+ ($p < 0.001$). Regarding occupation, the HBsAg–/HBV DNA+group had fewer students and more office workers ($p < 0.001$). The proportion of first-time blood donors was higher

in the HBV-infected group ($p < 0.05$), especially in the HBsAg+group, in which 98.9% (269/272) were first-time blood donors. ABO blood group and ethnicity showed no differences among the three groups ($p = 0.8$, and $p = 0.776$, respectively).

Results of 182 NAT R/HBsAg N/N samples

After screening for HBsAg and HBV DNA, 182 donors were included in the NAT R/HBsAg N/N group. Table 2 presents the results of the study. The proportion of HBV infections was 62.09% (113/182), of which 38.46% (70/182) were HBV+, 22.53% (41/182) were occult HBV infections (OBI), 0.55% (1/182) were chronic HBV infections, and 0.55% (1/182) were hepatitis B WPI. Notably, 37.91% (69/182) were false positives.

Results of 18 NAT R/HBsAg N/R samples

After HBsAg and HBV DNA screening, 18 donors were assigned to the NAT R/HBsAg N/R group. The confirmatory results are presented in Table 3. The proportion of patients with HBV infection was 94.44% (17/18), of which 50% (9/18) were OBI, 27.78% (5/18) were chronic HBV infections, and 16.67% (3/18) were HBV+. Notably, only 5.56% (1/18) were false positives. Interestingly, the test results for reagent 1 were negative for all 18 blood donors.

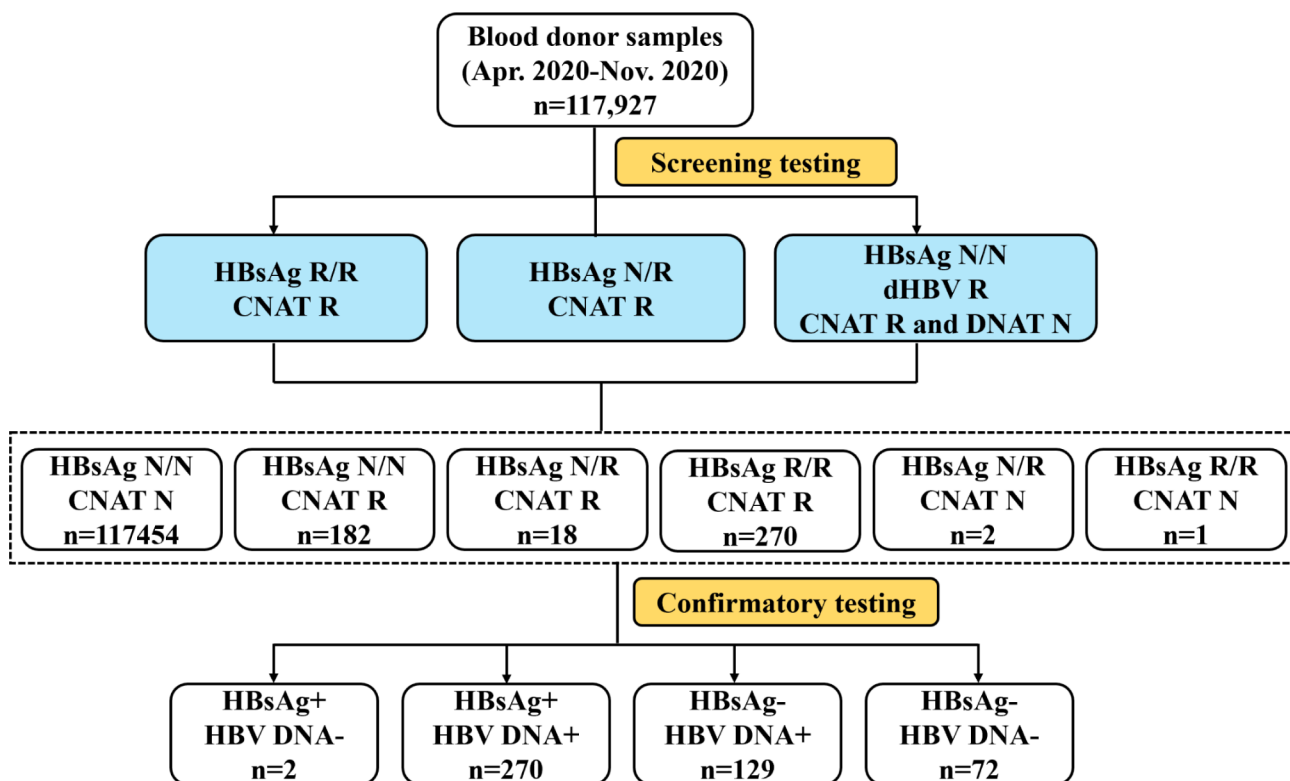


Fig. 2 Flow chart of the study. +: positive; -: negative; R: reactive; N: nonreactive

Table 1 Characteristics of enrolled donors(*n*=473)

| | All | | HBsAg+ | | HBsAg-/DNA+ | | HBsAg-/DNA- | | p-value |
|--|-------|---------|--------|---------|-------------|---------|-------------|---------|---------|
| | n=473 | | n=272 | | n=129 | | n=72 | | |
| | n | rate(%) | n | rate(%) | n | rate(%) | n | rate(%) | |
| Sex | | | | | | | | | < 0.001 |
| Male | 309 | (65.33) | 178 | (65.44) | 92 | (71.32) | 39 | (54.17) | |
| Female | 164 | (34.67) | 94 | (34.56) | 37 | (28.68) | 33 | (45.83) | |
| Age | | | | | | | | | < 0.001 |
| 18–25 | 61 | (12.90) | 47 | (17.28) | 8 | (6.20) | 6 | (8.33) | |
| 26–35 | 91 | (19.24) | 64 | (23.53) | 14 | (10.85) | 13 | (18.06) | |
| 36–45 | 102 | (21.56) | 49 | (18.01) | 38 | (29.46) | 15 | (20.83) | |
| > 46 | 219 | (46.3) | 112 | (41.18) | 69 | (53.49) | 38 | (52.78) | |
| ABO blood type | | | | | | | | | 0.8 |
| A | 151 | (31.92) | 93 | (34.19) | 39 | (30.23) | 19 | (26.39) | |
| B | 106 | (22.41) | 61 | (22.43) | 28 | (21.71) | 17 | (23.61) | |
| O | 174 | (36.79) | 90 | (33.09) | 54 | (41.86) | 30 | (41.67) | |
| AB | 42 | (8.88) | 28 | (10.29) | 8 | (6.20) | 6 | (8.33) | |
| Ethnicity | | | | | | | | | 0.776 |
| Han | 457 | (96.62) | 260 | (95.59) | 126 | (97.67) | 71 | (98.61) | |
| Non-Han | 14 | (2.96) | 11 | (4.04) | 2 | (1.55) | 1 | (1.39) | |
| Missing data | 2 | (0.42) | 1 | (0.37) | 1 | (0.78) | 0 | (0.00) | |
| Number of blood donations | | | | | | | | | < 0.001 |
| first time | 397 | (83.93) | 269 | (98.90) | 86 | (66.67) | 42 | (58.33) | |
| repeat | 76 | (16.07) | 3 | (1.10) | 43 | (33.33) | 30 | (41.67) | |
| Occupation | | | | | | | | | 0.005 |
| Student | 53 | (11.21) | 45 | (16.54) | 4 | (3.10) | 4 | (5.56) | |
| Freelancer | 22 | (4.65) | 7 | (2.57) | 10 | (7.75) | 5 | (6.94) | |
| Office worker | 71 | (15.01) | 42 | (15.44) | 22 | (17.05) | 7 | (9.72) | |
| Worker | 40 | (8.46) | 22 | (8.09) | 13 | (10.08) | 5 | (6.94) | |
| Civil worker /teacher/healthcare worker/Military personnel | 24 | (5.07) | 7 | (2.57) | 8 | (6.20) | 9 | (12.50) | |
| Farmer | 41 | (8.67) | 23 | (8.46) | 14 | (10.85) | 4 | (5.56) | |
| Others | 212 | (44.82) | 122 | (44.85) | 54 | (41.86) | 36 | (50.00) | |
| Missing data | 10 | (2.11) | 4 | (1.47) | 4 | (3.10) | 2 | (2.78) | |

Table 2 182 NAT R/HBsAg N/N sample results

| Results | n | Rate (%) |
|-------------------------------|-----|----------|
| HBV+ | 70 | 38.46 |
| False reactivity | 69 | 37.91 |
| OBI | 41 | 22.53 |
| Chronic hepatitis B infection | 1 | 0.55 |
| hepatitis B WPI | 1 | 0.55 |
| Total | 182 | |

+: positive; OBI: occult HBV infection; WPI: window period infection (WPI).

Results of 270 NAT R/HBsAg R/R samples

After the validation of HBsAg and HBV DNA, 270 donors were included in the NAT R/HBsAg R/R group, and the particular results are listed in Table 4. It is worth noting that all HBsAg confirmation results were negative, even though HBV DNA was positive, and four samples were positive for OBI.

Results of 72 HBV DNA-/HBsAg- (false reactivity) samples

After validation of HBsAg and HBV DNA, 72 donors were included in the HBV DNA-/HBsAg- group, and

the results are listed in Tables 5 and 6. Of the false positives, 95.83% (69/72) were from the NAT R/HBsAg N/N group, 58.33% (42/72) were first-time blood donors, and 1.39% (1/72) had more than 20 blood samples.

Discussion

In China, donors are routinely screened for HBsAg using ELISA twice plus NAT; however, due to the transient nature of HBV infection, the residual risk of infection remains high [11, 12]. To develop evidence-based, efficient, and safe screening strategies to reduce the relative risk of HBV infection, it is essential to obtain information regarding the prevalence, incidence, and associated demographic characteristics of blood donors.

A total of 117,927 samples were tested in this study. The prevalence of HBV was 0.4% (473/117927) in Chongqing, which was higher than that in Hefei (0.13%), Changzhi (0.16%), and Fujian (0.25%) [10]. HBV positivity was significantly higher in men than in women, which is in line with the HBV profile of the general population or the blood donor population abroad [13, 14]. This may

Table 3 18 NAT R/HBsAg N/R sample results

| Num. | Reagent 1 (S/CO) | Reagent2 (S/CO) | Results |
|------|------------------|-----------------|-------------------------------|
| 1 | 0.171 | 0.8 | OBI |
| 2 | 0.181 | 1.462 | OBI |
| 3 | 0.581 | 1.215 | OBI |
| 4 | 0.371 | 0.83 | OBI |
| 5 | 0.229 | 0.854 | OBI |
| 6 | 0.152 | 0.877 | OBI |
| 7 | 0.048 | 0.897 | OBI |
| 8 | 0.333 | 0.966 | OBI |
| 9 | 0.381 | 1.088 | OBI |
| 10 | 0.343 | 1.047 | Chronic hepatitis B infection |
| 11 | 0.486 | 1.154 | Chronic hepatitis B infection |
| 12 | 0.619 | 1.225 | Chronic hepatitis B infection |
| 13 | 0.695 | 0.983 | Chronic hepatitis B infection |
| 14 | 0.21 | 1.569 | Chronic hepatitis B infection |
| 15 | 0.105 | 0.804 | HBV+ |
| 16 | 0.21 | 0.815 | HBV+ |
| 17 | 0.229 | 0.991 | HBV+ |
| 18 | 0.514 | 1.029 | HBV- |

+: positive; -: negative

Table 4 Particular results of 270 NAT R/HBsAg R/R samples

| Reagent 1 (S/CO) | Reagent2 (S/CO) | HBsAg Confirmation results | HBV DNA Confirmation results | Results |
|------------------|-----------------|----------------------------|------------------------------|---------|
| 1.076 | 2.025 | - | + | OBI |
| 1.2 | 1.62 | - | + | OBI |
| 11.17 | 6.51 | - | + | HBV+ |
| 0.848 | 1.17 | - | + | OBI |
| 1.714 | 4.036 | - | + | OBI |

+: positive; -: negative

Table 5 Screening results of 72 false reactivity samples

| CNAT | HBsAg | n | Rate (%) |
|-------|-----------|----|----------|
| NAT R | HBsAg N/N | 69 | 95.83 |
| NAT N | HBsAg N/R | 2 | 2.78 |
| NAT R | HBsAg N/R | 1 | 1.39 |
| Total | | 72 | |

Table 6 Number of blood donations of 72 false reactivity samples

| Number of blood donations (Times) | n | Rate (%) |
|-----------------------------------|----|----------|
| 1 | 42 | 58.33 |
| 2~5 | 21 | 29.17 |
| 6~10 | 5 | 6.94 |
| 11~20 | 3 | 4.17 |
| >20 | 1 | 1.39 |
| Total | 72 | |

be related to factors such as greater male socialization, interpersonal opportunities, exposure to pathogens, and unsafe sexual practices, such as male–male sex. HBV positivity was the lowest in the 18–25 years age group

and highest in the 46+ years age group. This is consistent with reports from other blood banks [15, 16]. This is because, since the introduction of the National Hepatitis B Vaccination Programme in 1992, the prevalence of HBV infection has been gradually decreasing [17–19]. The prevalence in the general population of China fell from 9.8 to 7.2% after the introduction of HBV vaccination [20]. Public servants (civil servants, doctors, and teachers) have the lowest HBV positivity rate, coinciding with blood donors' literacy levels. Higher levels of education are associated with better knowledge of infectious disease prevention and control and a lower risk of HBV infection. First-time donors are more likely to be HBV-positive than repeat donors, suggesting that maintaining a stable population of donors may be useful in reducing the risk of transfusion-transmitted HBV. This finding suggests that first-time blood donors should be recruited from low-risk groups. Pre-donation counseling and initial screening for HBsAg before donation can improve blood safety. Furthermore, a long-term mechanism must be established to retain repeat donors through a variety of effective measures and in promoting the healthy and stable development of the blood donor workforce.

Among the 182 NAT R/HBsAg N/N samples, 62.09% (113/182) were found to be infected with HBV, indicating those that blood screening by ELISA alone may be missed. NAT is efficient in detecting low viral load samples to ensure the safety of blood supplies, and negative ELISA results may be partly due to OBI. OBI is defined as the presence of replication-competent HBV DNA in the liver and/or HBV DNA in the blood of persons testing negative for HBsAg by currently available assays, with or without detectable anti-HBc or anti-HBs [21]. The prevalence of OBI in Chinese blood donors is approximately 0.94 per 1000 [22, 23]. Some studies have reported that OBI donors with a low viral load may be missed, resulting in transfusion-transmitted infection with HBV [11, 24–26]. The residual risk of HBV transfusion is closely related to the prevalence of HBV infection, the population of blood donors, and screening strategies in place. The window period, low concentration load, OBI, and immunological quiescence of viral strains lead to difficulties in HBV detection and transfusion transmission [27]. Of the 182 NAT R/HBsAg N/N samples, 37.91% (69/182) were false positives. Unfortunately, blood donors in China who are blocked by a positive nucleic acid test are permanently barred from donating. Therefore, quality control of nucleic acid laboratories should be managed as much as possible to avoid contamination.

Among the 18 NAT R/HBsAg N/R samples, 94.44% (17/18) tested positive for HBV. Interestingly, the reagent 1 test was negative in all samples. The results show that the detection efficiency of reagent 1 was lower than that of reagent 2. The S/CO ratio of 55.6% (10/18) in reagent

2 ranged from 0.8–1. If the cutoff was set to 1, some of the samples would have been considered non-reactive. The gray area setting is important; if it is too low, there will be an increase in the amount of blood waste. However, owing to a combination of factors, such as testing personnel, equipment, reagents, and testing capacity, each laboratory should set its gray zone. It is also recommended that donors not be blocked by gray zone results. They can have a blood donation appointment at the end of their blood donation interval, and if the test results are acceptable, they can donate. This helps retain donors and ensure blood safety.

Of the 270 NAT R/HBsAg R/R samples, five were ELISA/NAT reactive and chemiluminescent negative for HBsAg. This is because five blood donors had an OBI. These findings suggested that different tests should be used for blood screening. NAT and ELISA complement each other, and when used together, further reduce the risk of residual HBV. Blood organizations should evaluate the detection performance of the reagents currently in use to ensure blood safety. It has been reported that the current residual risk of TT-HBV infection is still high (overall 56.53 per 10⁵ person–years) despite the introduction of more sensitive blood screening tests [10]. Continuous monitoring of the risk of residual transfusion-transmitted infections is essential to ensure safe blood management.

Conclusion

Our study demonstrates that HBV infection is still present in blood donors in Chongqing, despite a large-scale vaccination campaign initiated more than 30 years ago. This is probably due to the high rate of HBV infection before vaccination, silent development of latent HBV persistence, and establishment of OBI [28, 29]. Double ELISA and single NAT can effectively prevent HBV leakage and improve blood safety; however, the false reactivity of NAT is high, and laboratory quality control should be improved. First-time donors have a high rate of HBV transplant failure; therefore, donors should be retained and recruited from low-risk groups. Regular analysis of laboratory serology and NAT results can help assess the reagent detection efficiency and provide a basis for reagent selection and screening strategies.

Abbreviations

| | |
|----------|--|
| HBV | Hepatitis B virus |
| ELISA | Enzyme-linked immunosorbent assay |
| HBsAg | Hepatitis B surface antigen |
| NAT | Nucleic acid testing |
| anti-HBc | Hepatitis B core antigen |
| HCV | Hepatitis C virus |
| HIV | Human immunodeficiency virus |
| OBI | Occult HBV infection |
| CNAT | HBV/HIV/HCV combined nucleic acid test |
| DNAT | HBV/HIV/HCV differential nucleic acid test |

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Author contributions

Lan Wei: Sample collection, Blood donor follow-up, Funding acquisition, Formal analysis, Data Curation, Writing-Original Draft. Min Chen: Sample collection, Blood donor follow-up. Fang Wang: Blood donor follow-up. Meijun Li: Investigation. Dong Liu: Sample Collection. Chengbing Xie: Supervision. Dongyan Yang: Review and Editing. Siyang Wen: Data curation and Writing. Yongzhu Xu: Project administration. All authors contributed to the article and approved the submitted version.

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Data availability

Raw data in support of the conclusions in the article will be available from the authors without undue restriction.

Declarations

Ethics approval and consent to participate

This study was reviewed and approved by the Medical Ethics Committee of the Chongqing Blood Center. Participants provided written informed consent to participate in this study. Additionally, written informed consent was obtained from the individual(s) to publish any potentially identifiable images or data in this article.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Olotu AA, Oyelese AO, Salawu L, Audu RA, Okwuraiwe AP, Aboderin AO. Occult Hepatitis B virus infection in previously screened, blood donors in Ile-Ife, Nigeria: implications for blood transfusion and stem cell transplantation. *Virology*. 2016;13:76.
2. Argirion I, Pfeiffer RM, Lam TK, O'Brien TR, Yu K, McGlynn KA, Petrick JL, Pinto L, Chen CJ, Lee MH, et al. Association between immunologic markers and cirrhosis in individuals with chronic hepatitis B. *Sci Rep*. 2021;11(1):21194.
3. Hughes E, Bassi S, Gilbody S, Bland M, Martin F. Prevalence of HIV, Hepatitis B, and hepatitis C in people with severe mental illness: a systematic review and meta-analysis. *Lancet Psychiatry*. 2016;3(1):40–8.
4. Yin F, Xie Y, Fan H, Zhang J, Guo Z. Mutations in hepatitis B virus polymerase are associated with the postoperative survival of hepatocellular carcinoma patients. *PLoS ONE*. 2017;12(12):e0189730.
5. Zbinden A, Ries J, Redli PM, Shah C, Glauser A, Goslings D, Huzly D, Böni J, Gottschalk J, Frey BM. Prevalence of Occult Hepatitis B Virus infection in

- blood donors with negative ID-NAT in Switzerland. *Transfus Med Hemother*. 2022;49(6):338–45.
6. Levicnik-Stezinar S, Rahne-Potokar U, Candotti D, Lelie N, Allain JP. Anti-HBs positive occult hepatitis B virus carrier blood infectious in two transfusion recipients. *J Hepatol*. 2008;48(6):1022–5.
 7. Candotti D, Boizeau L, Laperche S. Occult Hepatitis B infection and transfusion-transmission risk. *Transfus Clin Biol*. 2017;24(3):189–95.
 8. Candotti D, Allain JP. Transfusion-transmitted hepatitis B virus infection. *J Hepatol*. 2009;51(4):798–809.
 9. Niederhauser C. Reducing the risk of hepatitis B virus transfusion-transmitted infection. *J Blood Med*. 2011;2:91–102.
 10. Li L, Han T, Zang L, Niu L, Cheng W, Lin H, Li KY, Cao R, Zhao B, Liu Y, et al. The current incidence, prevalence, and residual risk of hepatitis B viral infections among voluntary blood donors in China. *BMC Infect Dis*. 2017;17(1):754.
 11. Li W, Gao Z, Yang C, Li J, Li L, Lv R, Liu Z. The estimation of prevalence, incidence, and residual risk of transfusion-transmitted human hepatitis B infection from blood donated at the Anhui blood center, China, from 2009 to 2011. *PLoS ONE*. 2013;8(9):e73472.
 12. Centers for Disease C, Prevention. Progress in Hepatitis B prevention through universal infant vaccination—China, 1997–2006. *MMWR Morb Mortal Wkly Rep*. 2007;56(18):441–5.
 13. Wang H, Men P, Xiao Y, Gao P, Lv M, Yuan Q, Chen W, Bai S, Wu J. Hepatitis B infection in the general population of China: a systematic review and meta-analysis. *BMC Infect Dis*. 2019;19(1):811.
 14. De Brier N, Koc OM, De Buck E, Muylaert A, Nevens F, Vanbrabant M, Vandelooy J, Van Remoortel H, Robaey G, Compennolle V. Hepatitis B virus prevalence in first-time blood donors in Flanders, Belgium: impact of universal vaccination and migration. *Transfusion*. 2021;61(7):2125–36.
 15. Deng X, Liu D, Delcourt MP, Gao H, Zhou L, Candotti D. No Hepatitis Delta Virus Seropositivity among blood donors with overt and Occult Hepatitis B Infection in Dalian, Liaoning Province, China. *Viruses* 2023, 15(7).
 16. Chang L, Yan Y, Ji H, Sun H, Jiang X, Lu Z, Wang L, Group H. Low seroprevalence of Hepatitis delta virus co-infection in hepatitis B virus-infected blood donors in China: a multicenter study. *Front Microbiol*. 2022;13:992817.
 17. Tang X, Allain JP, Wang H, Rong X, Chen J, Huang K, Xu R, Wang M, Huang J, Liao Q, et al. Incidence of hepatitis B virus infection in young Chinese blood donors born after mandatory implementation of neonatal hepatitis B vaccination nationwide. *J Viral Hepat*. 2018;25(9):1008–16.
 18. Ye X, Li T, Yu B, Zeng J, Shi Y, Xie H, Branch DR, Loriani M, Li B, Chen L. The high prevalence of occult hepatitis B infections among the partners of chronically infected HBV blood donors emphasizes the potential residual risk to blood safety. *J Med Virol*. 2023;95(8):e29006.
 19. Liu J, Liang W, Jing W, Liu M. Countdown to 2030: eliminating hepatitis B disease, China. *Bull World Health Organ*. 2019;97(3):230–8.
 20. Liang X, Bi S, Yang W, Wang L, Cui G, Cui F, Zhang Y, Liu J, Gong X, Chen Y, et al. Evaluation of the impact of hepatitis B vaccination among children born during 1992–2005 in China. *J Infect Dis*. 2009;200(1):39–47.
 21. Mendoza SG, Zerpa A, Velazquez E, Schwarzenberg A, Hynd B, Kashyap ML, Laskarzewski P, Glueck CJ. Sex hormones, lipids, lipoprotein cholesterols, and apolipoproteins in normal and obese subjects: atherogenic relationships. *Int J Obes*. 1986;10(6):427–41.
 22. Zhang L, Chang L, Laperche S, Ji H, Zhao J, Jiang X, Wang L, Candotti D. Occult HBV infection in Chinese blood donors: role of N-glycosylation mutations and amino acid substitutions in S protein transmembrane domains. *Emerg Microbes Infect*. 2019;8(1):1337–46.
 23. Liu GC, Sui GY, Liu GY, Zheng Y, Deng Y, Gao YY, Wang L. A bayesian meta-analysis on prevalence of hepatitis B virus infection among Chinese volunteer blood donors. *PLoS ONE*. 2013;8(11):e79203.
 24. Raimondo G, Locarnini S, Pollicino T, Levrero M, Zoulim F, Lok AS. Taormina Workshop on Occult HBVIFM: update of the statements on biology and clinical impact of occult hepatitis B virus infection. *J Hepatol*. 2019;71(2):397–408.
 25. Candotti D, Assennato SM, Laperche S, Allain JP, Levicnik-Stezinar S. Multiple HBV transfusion transmissions from undetected occult infections: revising the minimal infectious dose. *Gut*. 2019;68(2):313–21.
 26. Weusten J, van Drimmelen H, Vermeulen M, Lelie N. A mathematical model for estimating residual transmission risk of occult hepatitis B virus infection with different blood safety scenarios. *Transfusion*. 2017;57(3pt2):841–9.
 27. Ma Q, Wang Y. Comprehensive analysis of the prevalence of hepatitis B virus escape mutations in the major hydrophilic region of surface antigen. *J Med Virol*. 2012;84(2):198–206.
 28. Stroffolini T, Stroffolini G, Pollicino T. Five decades of HBV Infection in Italy: a continuous challenge. *Biology (Basel)* 2023, 12(8).
 29. Saitta C, Pollicino T, Raimondo G. Occult Hepatitis B Virus infection: an update. *Viruses* 2022, 14(7).

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