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Prevalence of hepatitis B and syphilis co-infection and its association with ABO/Rh blood groups among Sudanese blood donors, Sudan

Abstract

Background: Transfusion-transmissible infections (TTIs) remain a major challenge to blood safety in low-resource settings. This study assessed the prevalence of Hepatitis B virus (HBV) and Syphilis co-infection and its association with ABO/Rh blood groups among Sudanese blood donors.

Methods: A cross-sectional study was conducted at the Central Blood Bank in Khartoum State from January to August 2015. A total of 35,385 donor samples were screened for HBV surface antigen (HBsAg) and Syphilis antibodies using ELISA. ABO and Rh blood grouping were performed by standard agglutination.

Results: HBV mono-infection was detected in 3.6% (1,274/35,385) of donors, Syphilis mono-infection in 2.6% (905/35,385), and HBV–Syphilis co-infection in 0.2% (76/35,385). All co-infected donors were male, with the majority aged 31–40 years (60.5%). Blood group O Rh-positive was most frequent among co-infected individuals (39.5%), followed by A Rh-positive (35.5%) and B Rh-positive (19.8%). Critically, all co-infected donors were family replacement donors.

Conclusion: HBV–Syphilis co-infection is uncommon among Sudanese blood donors but remains a transfusion risk. The predominance among O Rh-positive donors suggests a possible biological association that requires further investigation. Strengthening voluntary donation and advanced screening methods is essential to enhance transfusion safety.

Keywords: Hepatitis B, syphilis, co-infection, blood donors, blood group, Sudan

Volume 13 Issue 3 - 2025

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Received: October 24, 2025 | **Published:** November 5, 2025

Introduction

Transfusion-transmissible infections (TTIs) continue to pose a significant threat to blood safety in low-resource settings. Despite improvements in donor screening and laboratory testing, TTIs remain a major public health concern, especially in developing countries where infrastructure and diagnostic resources are limited.[1,2] According to the World Health Organization (WHO), millions of blood donations are collected annually worldwide, with a significant proportion originating from low- and middle-income countries where safety standards can be variable.[3] These nations face persistent challenges in ensuring blood safety due to insufficient screening capacity, irregular donor follow-up, and dependence on family replacement donation.[4,5] Hepatitis B virus (HBV) infection remains one of the most common TTIs, affecting over 290 million people globally and accounting for nearly 820,000 deaths annually, mainly from cirrhosis and hepatocellular carcinoma.[6,7] Syphilis, caused by *Treponema pallidum*, though less prevalent, continues to represent a transfusion risk when donor screening is incomplete.[8] Both HBV and Syphilis share similar routes of transmission—through infected blood, sexual contact, and from mother to child—thus, co-infection among blood donors is possible and may amplify transfusion-related risks.[9,10] Recent studies have highlighted ongoing TTI burdens across Africa and Asia. For example, a 2023 Pakistani study reported HBsAg and Syphilis seroprevalence rates of 2.98% and 0.41%, respectively, among blood donors.[11] Similarly, a 2024 study in Bangladesh reported HBsAg positivity at 1.2% among donors,[12] while a 2025 investigation in Somaliland found an HBV-Syphilis co-infection prevalence of 0.3%.[13] These findings underscore that TTIs remain an ongoing concern despite technological advances in screening and donor selection. In Sudan, transfusion safety remains a challenge due to reliance on replacement donors and limited use of advanced testing methods such as nucleic acid amplification tests (NAT). Previous studies have shown moderate endemicity of HBV among Sudanese blood donors, with prevalence rates ranging from 3.6% to 6.1%.[14-16] The possible association between blood group antigens and infection susceptibility has been proposed in several infectious diseases, including hepatitis viruses and bacterial pathogens.[17,18] Blood group antigens may act as receptors or co-receptors for viral attachment, influence immune recognition, or reflect genetic linkages affecting host susceptibility. However, the relationship between ABO/Rh blood groups and HBV-Syphilis co-infection has not been fully elucidated in Sudanese populations. Therefore, this study aimed to determine the prevalence of HBV and Syphilis co-infection among Sudanese blood donors and to evaluate its possible association with ABO/Rh blood group distribution. This research provides updated evidence to enhance transfusion safety policies and deepen understanding of the epidemiological patterns of TTIs in Sudan.

Methodology

Study design and setting

A cross-sectional descriptive study was conducted at the Central Blood Bank in Khartoum State, Sudan, from January to August 2015. The Central Blood Bank is the main blood collection and distribution facility in Sudan, serving multiple hospitals in the capital and nearby regions.

Study population

The study included a total of 35,385 apparently healthy blood donors who met the eligibility criteria established by the Sudanese National Blood Transfusion Services. Donors were selected consecutively during the study period to minimize selection bias.

Inclusion and exclusion criteria

Inclusion criteria: Individuals aged between 18–60 years, body weight ≥50 kg, hemoglobin concentration ≥12.5 g/dL, and clinically healthy at the time of donation.

Exclusion criteria: Donors who refused consent, presented signs of active infection, or had incomplete demographic or serological data were excluded from the analysis.

Sample collection and processing

Approximately 5 mL of venous blood was collected aseptically from each donor into plain sterile tubes. After clotting, samples were centrifuged at 3,000 rpm for 5 minutes, and serum was separated and stored at 2–8 °C until testing.

Table 1 Distribution of Infections among Blood Donors

Infection Status	Frequency	Percent (%)
HBV positive	1,274	3.6
Syphilis positive	905	2.6
HBV + Syphilis co-infection	76	0.2
Negative	33,130	93.6
Total	**35,385**	**100.0**

Table 2 Distribution of Co-infected Donors by Blood Group

Blood Group	Frequency	Percent (%)
A+	27	35.5
A–	1	1.3
O+	30	39.5
B+	15	19.8
AB+	3	3.9
Total	**76**	**100.0**

Serological testing

All donor samples were screened for Hepatitis B surface antigen (HBsAg) and *Treponema pallidum* antibodies using commercial enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's protocols.

- **HBsAg detection:** *Hepanostika HBsAg ELISA Kit* (BioMérieux, France)
- **Syphilis detection:** *Syphilis ELISA Kit* (DIA.PRO Diagnostic Bioprobes, Italy)

Reactive samples were retested for confirmation. Donors who tested positive for both HBsAg and Syphilis antibodies were classified as *co-infected*.

Blood group determination

ABO and Rhesus (Rh) blood grouping was performed using the standard tube agglutination method with commercial antisera (Anti-A, Anti-B, and Anti-D).

Statistical analysis

All data were entered and analyzed using the Statistical Package for the Social Sciences (SPSS) version 25. Descriptive statistics were used to summarize categorical variables as frequencies and percentages. Continuous variables, such as donor age, were expressed as mean ± standard deviation (SD). The frequency percentages of co-infected donors by blood group were calculated using the following formula: Percentage = (Number of co-infected donors in each blood group / Total number of co-infected donors) × 100. The Chi-square test was employed to evaluate the association between categorical variables (e.g., infection status and blood group type). A p-value < 0.05 was considered statistically significant. Multivariate logistic regression analysis was performed to identify independent predictors of HBV–Syphilis co-infection after adjusting for age, sex, and donation type.

Results

A total of 35,385 blood donor samples were analyzed during the study period. The mean age of the donors was 34.8 ± 7.6 years (range: 18–60 years). Among the study population, 1,274 (3.6%) tested positive for Hepatitis B virus (HBV), 905 (2.6%) for Syphilis, and 76 (0.2%) were co-infected with both pathogens. All co-infected donors were male (100%), and the highest proportion of co-infection was observed among donors aged 31–40 years (60.5%). **A significant finding was that all 76 co-infected donors (100%) were family replacement donors,** with no co-infected individuals detected among voluntary donors. The mean age of donors infected with HBV only was 33.9 ± 8.2 years, while those infected with Syphilis only had a mean age of 35.4 ± 6.8 years. The mean age of co-infected donors was slightly higher at 36.1 ± 6.5 years. The O Rh-positive blood group was the most frequent among co-infected donors (39.5%), followed by A Rh-positive (35.5%) and B Rh-positive (19.8%). Statistical analysis revealed a significant association between co-infection status and blood group type ($p < 0.05$). However, there was no significant relationship between co-infection and donor age group ($p > 0.05$).

Discussion

The present study investigated the frequency of co-infection between Hepatitis B virus (HBV) and Syphilis among Sudanese blood donors and its possible association with ABO/Rh blood group distribution. The overall co-infection rate observed (0.2%) indicates a relatively low prevalence among blood donors in Khartoum State. However, even a small percentage of co-infection remains clinically important since both HBV and Syphilis are transfusion-transmissible infections (TTIs) capable of causing severe health consequences in recipients.[19,20] This finding is consistent with recent studies from Africa and Asia. For instance, Hassan et al.[13] in Somaliland reported a co-infection prevalence of 0.3%,[13] while a 2023 Pakistani study demonstrated HBV and Syphilis prevalence of 2.98% and 0.41% respectively among donors.[11] These results indicate that although screening methods have improved, TTIs remain a persistent challenge in developing countries due to limited resources, poor donor education, and the high proportion of replacement donors.

The prevalence of HBV mono-infection in this study (3.6%) aligns with national data from recent Sudanese studies showing moderate endemicity of HBV, ranging from 3% to 6% among blood donors.[14,16,21] A slight decline compared to earlier data (6.1%) may reflect improved awareness and better screening strategies at blood banks. The Syphilis prevalence (2.6%) is comparable to previous reports from Ethiopia (2.9%) and Nigeria (2.5%),[22,23] yet remains higher than reports from Egypt (0.9%).[24] Variations between countries may be explained by differences in population behavior, risk exposure, and screening sensitivity. The observed male predominance (100%) and concentration of cases in the 31–40-year age group agree with studies across sub-Saharan Africa, where infection risk is higher among adult males due to increased exposure to occupational hazards and socio-behavioral factors such as multiple sexual partnerships and limited STI awareness.[25,26] Similar trends were reported in Sudanese and Ethiopian studies, where men were more likely to be infected with HBV or Syphilis than women.[27]

A pivotal finding of this study is that all co-infected donors were family replacement donors. This strongly supports earlier reports that replacement donors carry a higher risk of transfusion-transmissible infections than voluntary, non-remunerated donors.[5,28] This may be attributed to the pressure or emotional obligation involved in family donation, which might lead potential donors to conceal risk factors or health information during the screening process. The predominance

of co-infection among O Rh-positive donors (39.5%) may suggest an underlying biological association. ABO and Rh blood group antigens are known to influence susceptibility to several infectious agents, including hepatitis viruses and *Treponema pallidum*, possibly by acting as receptors for pathogen attachment or by modulating immune response pathways.[17,29] Studies by Kumar et al.[30] also reported higher HBV prevalence among O Rh-positive individuals, consistent with the current findings.[30] In contrast, Joshi and Ghimire[31] found no statistically significant association between blood group type and HBV-Syphilis co-infection.[31] These conflicting results highlight the need for further molecular and immunogenetic research to clarify this potential relationship in Sudanese populations.

Although the study data were collected in 2015, they remain relevant, as Sudan has not implemented major nationwide blood safety reforms or national TTI surveys since that period. Furthermore, the current findings are consistent with the trends reported in recent regional and global studies conducted between 2022 and 2025, supporting their continued validity.[11-13,32] Overall, the low co-infection rate observed reflects the partial success of current blood safety measures, yet underscores the need for continuous vigilance. **The exclusive identification of co-infection among family replacement donors provides a clear directive for public health policy.** Strengthening voluntary non-remunerated donation programs, public health education, and the adoption of advanced screening methods—such as nucleic acid testing (NAT)—are essential to further reduce transfusion risks in Sudan.

Study limitations

Diagnostic accuracy was limited by the use of ELISA without confirmatory molecular methods such as nucleic acid testing (NAT). Future research should integrate molecular and genetic approaches to validate these findings and explore host susceptibility mechanisms.

Conclusion

The study found that HBV–Syphilis co-infection is rare (0.2%) among Sudanese blood donors but remains a threat to transfusion safety. Most cases were observed in male, family replacement donors with O Rh-positive blood, suggesting potential epidemiological links. Despite using 2015 data, the findings highlight the need for national surveillance, molecular diagnostics, and strengthened preventive measures to ensure safe blood transfusion.

Recommendations

a. Maintain mandatory screening for Hepatitis B and Syphilis in all blood donations across Sudanese blood banks.

b. Introduce advanced diagnostic tools, including nucleic acid testing (NAT), to improve detection accuracy and reduce window-period infections.

c. Promote voluntary non-remunerated blood donation (VNRBD) through nationwide awareness and educational campaigns to replace family replacement donations.

d. Enhance pre-donation counseling and risk assessment to identify high-risk donors and reduce TTI prevalence.

e. Establish national data repositories and regular monitoring systems for transfusion-transmissible infections to guide evidence-based policy.

f. Conduct future molecular and immunogenetic studies to explore the potential relationship between ABO/Rh blood groups and susceptibility to HBV–Syphilis co-infection.

Acknowledgments

None.

Consent

The patient's written consent has been collected.

Ethical considerations

Ethical approval was obtained from the Research Ethics Committee of the Sudan Academy of Science, and permission was granted by the Central Blood Bank, Khartoum State. Written informed consent was obtained from all participants, and data confidentiality was strictly maintained throughout the study in accordance with the Declaration of Helsinki.

Sources of funding

No specific funding was received for this research.

Conflict of interest

The authors declare no conflicts of interest.

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Citation: Hamid MAM, Mohamed YM, Abuzeid N,et al. Prevalence of hepatitis B and syphilis co-infection and its association with ABO/Rh blood groups among Sudanese blood donors, Sudan. *J Microbiol Exp.* 2025;13(3):113–116. DOI: 10.15406/jmen.2025.13.00442