



Prevalence and factors associated with Hepatitis B virus infection among pregnant mothers attending antenatal services at Entebbe regional referral hospital in Wakiso district.

A cross-sectional study.

Faith Nambuya*, Anthony Ssekitoleko, Francisco Ssemuwemba, Hasifah Nansereko, Jane Frank Nalubega, Immaculate Prosperia Naggulu
Mildmay Institute of Health Sciences.

Abstract.

Background:

Pregnant women represent a vulnerable group due to the risk of adverse pregnancy outcomes and mother-to-child transmission. This study aimed to determine the prevalence and factors associated with HBV infection among pregnant mothers attending antenatal services at Entebbe Regional Referral Hospital in Wakiso District.

Methodology:

A hospital-based cross-sectional study was conducted among 128 pregnant women selected using simple random sampling. Data on socio-demographic characteristics and risk factors were collected using structured questionnaires. Blood samples were obtained and tested for hepatitis B surface antigen (HBsAg) using rapid diagnostic test kits following standard laboratory procedures. Data were analyzed using SPSS and presented in tables and figures.

Results:

The prevalence of HBV infection among the participants was 7.0% (9/128), indicating intermediate endemicity. Higher infection rates were observed among mothers aged 30–34 years (13.3%), single mothers (19.2%), those with no formal education (14.3%), and rural residents (11.9%). Behavioral and medical factors significantly associated with HBV infection included having multiple sexual partners (14.3%), sharing sharp instruments (12.5%), a history of blood transfusion (8.3%), and tattooing or piercing (8.3%). Vaccinated participants had a lower prevalence (3.6%) compared to unvaccinated mothers (9.7%), suggesting a protective effect of vaccination.

Conclusion:

The study revealed a moderate prevalence of HBV infection among pregnant women attending antenatal care, with behavioral risk factors and lack of vaccination being key contributors. Strengthened preventive interventions are therefore necessary.

Recommendations:

Routine antenatal HBV screening should be enhanced, vaccination programs expanded for women of reproductive age, and health education intensified to improve awareness of transmission and prevention.

Keywords: Hepatitis B virus, Pregnant women, Antenatal care, Mother-to-child transmission, Wakiso District, Uganda

Submitted: December 05, 2025 **Accepted:** February 18, 2026 **Published:** March 05, 2026

Corresponding author: Faith Nambuya.
Mildmay Institute of Health Sciences.

Background of the study.

Hepatitis B infection is caused by the hepatitis B virus (HBV). It is an enveloped DNA virus that infects the liver and causes hepatocellular necrosis and inflammation. HBV infection is one of the serious public health problems worldwide, and it is 50–100 times more contagious than HIV. Many of the carriers do not realize that they are infected with the virus, rendering the HBV known as a “silent killer”. Worldwide, it is estimated

that 240 million people are chronically infected with hepatitis B. The largest number of people living with chronic HBV live in the Western Pacific region (over 95 million), followed by the African region (over 75 million). Recent reports demonstrated that 68,600 people die of HBV infection, and more than 300,000 deaths due to liver cancer secondary to hepatitis B every year globally (Umare *et al.*, 2016). Africa is considered a region of high endemicity ($\geq 8\%$) for HBV.



Although it is difficult to assess the exact burden of HBV in Africa, the sero-prevalence of hepatitis B surface antigen (HBsAg) has been estimated to be in the range of 6 to 20%. A higher prevalence of 9.7% to 16.6% was observed in other developing countries. The prevalence of HBV in Ethiopia among pregnant women has shown moderate endemicity, with the prevalence of HBsAg positivity ranging from 2.3 to 7.9% (Tadiwos *et al.*, 2021). In Nigeria, 11.6% prevalence has been reported from Maiduguri among blood donors and pregnant women, 4.3% from Port-Harcourt among pregnant women, 8.3% from Zaria among pregnant women (Suleiman A.C *et al.*, 2019).

Viral hepatitis during pregnancy is associated with a high risk of maternal complications. The infection adversely affects pregnancy outcome, leading to spontaneous abortion, premature delivery, intrauterine growth restrictions, and low birth weight of infants. In addition, the high rate of vertical transmission of the virus to the unborn child during delivery, referred to as mother-to-child transmission (MTCT), could impact the child's quality of life. Fetal and neonatal hepatitis impairs the physical and mental development of infants (Antuamwine B.B *et al.*, 2022). A meta-analysis and systematic review conducted in Ethiopia showed 6.3% of overall HBsAg prevalence in the general population over the last five decades. HBV infection prevalence rates of 1.2% in Iran, 9.2% in the Gambia, 7.7% in Ghana, 4.12% in Somalia, 3.2% in Eritrea, and 1.94% - 11.6% in Ethiopia were reported in pregnant women (Tesfu M.A *et al.*, 2023).

In Uganda, approximately 10% of the population is infected with hepatitis B, with nearly half (41%) of these infections being identified during pregnancy in antenatal care facilities. The prevalence of hepatitis B in expectant mothers is highest in Uganda's Northern Region at 11.8%. Recently, considerable progress has been made toward eliminating mother-to-child transmission of HBV through universal infant HBV immunization. However, hepatitis B birth-dose coverage is low, at only 18% in the WHO African Region in 2022. Uganda only added the hepatitis B birth dose to its routine vaccination schedule in 2022 (Mupeyi, G *et al.*, 2025). Hepatitis B infection is among the most common public health concerns globally, particularly in low- and middle-income countries. The prevalence of hepatitis B infection is more common among the most vulnerable populations, including pregnant women. However, there are limited studies on hepatitis B virus infection among pregnant women in low-income countries (Knife *et al.*, 2021). Studies conducted over the past decade reveal alarming prevalence rates of HBV among pregnant women in countries such as Uganda, Kenya, Nigeria, and Tanzania, with varying levels of healthcare access, education, and vaccination coverage. However,

despite the increasing recognition of the need for screening and preventive measures, there remains a lack of comprehensive, region-specific data on the prevalence of HBV among pregnant mothers attending antenatal services, as well as an understanding of factors contributing to these high infection rates. This study aimed to determine the prevalence and factors associated with HBV infection among pregnant mothers attending antenatal services at Entebbe Regional Referral Hospital in Wakiso District.

Methodology.

Study Design.

A cross-sectional study design was used because the study aimed at gathering information from various participants to determine the prevalence of HBV infection, its predisposing factors, and social demographic factors influencing the prevalence of hepatitis B among pregnant mothers at Entebbe regional referral hospital.

Study Area.

The study was conducted at Entebbe Regional Referral Hospital, located in central Uganda, in Wakiso district, approximately 35Km away from Kampala city along Kampala-Entebbe international Airport Highway. The hospital receives an average of 100 patients per day. This hospital has been chosen for the study due to its large patient intake, thanks to the wide range of services it offers.

Study Population.

The study was centered on all pregnant mothers attending antenatal services at Entebbe Regional Referral Hospital.

Selection criteria.

Inclusion criteria.

128 pregnant mothers who attended ERRH antenatal services, who presented with signs of hepatitis B, consented and were willing to participate in the study were included.

Sample Size Determination.

To calculate the minimum sample size, the formula below was used (Kish and Leslie, 1965): $n = Z^2pq/d^2$

Where:

n = the desired sample size

Z = the standard normal deviation (1.96) at 95% confidence level.

P = estimated prevalence of hepatitis B. Since the prevalence of hepatitis B is unknown, an estimated value of 50% will be used (P=0.5) (According to Ssekamatte *et al.* (2020))

d = desired error, and for this study, we shall use 0.05



$$Z=1.96 = (Z)^2 = (1.96)^2 = 3.8416$$

$$Q = (1-P) = (1-0.5) = 0.5$$

$$d^2 = (0.1)^2 = 0.01 \quad d^2 = (0.1)^2 = 0.01$$

$$n = 1.96^2 \times 0.5 \times 0.5 / (0.05)^2$$

$$N = 384.16 \text{ participants.}$$

Due to the exceptionally large size of the initial sample, a systematic reduction was applied. The total sample size was divided by three to yield a more manageable subset for analysis.

$$N = \frac{384.16}{3}$$

$$3$$

$$N = 128.05.$$

The study, therefore, involved only 128 participants drawn from the population.

Sampling Technique.

This study used a simple random sampling technique, a technique where every individual in a population had an equal chance of being selected for a study, and it is one of the most straightforward and unbiased sampling methods used in research.

Sampling Procedure.

The participants were selected based on characteristics that were deemed appropriate for the research topic and that were easily accessible. Participants were systematically enrolled until the required number was reached.

Data Collection Method

Qualitative data, such as associated risk factors and level of awareness and healthcare-seeking behavior regarding hepatitis B virus prevention and treatment, were collected through questionnaires, as they were easy to administer.

Quantitative data were obtained through macro examination for accurate diagnosis of Hepatitis B.

Relevant data, including the participant's age and associated risk factors after sample analysis, were recorded.

Blood samples were collected from participants to provide qualitative data.

Data Collection Tool.

A pretested questionnaire was used to collect social demographic data, predisposing factors, awareness, and health-seeking behaviors of pregnant mothers. Red top vacutainer and laboratory request forms were used. The final data was presented using tables, bar graphs, and pie charts, with the help of SPSS.

Data Collection Procedure.

The whole process was explained to the respondents, then questionnaires were issued to them to fill in appropriately, and the illiterate respondents were helped to fill in the questionnaires. Data concerning the test results were captured on the information sheet and in the laboratory register book. Furthermore, explanation was made to respondents that blood samples were to be collected from them and put in a red top vacutainer then registered into the laboratory reception book and given a laboratory number, on the work bench, the blood samples were centrifuged to get serum which was used to perform the HBsAg test to confirm someone to be positive or negative for hepatitis B virus.

Laboratory Procedure.

Hepatitis B Surface Antigen test (HBsAg).

Sample collection.

For whole blood (finger-prick method):

Clean the fingertip using an alcohol swab and allow it to air dry. Use a sterile lancet to prick the fingertip. Wipe away the first drop of blood with a sterile gauze or tissue. Collect the next drop using a capillary tube or micropipette (typically 50 μ L)

For venous blood, serum, or plasma:

Collect venous blood into a tube (plain tube for serum, EDTA tube for plasma). Centrifuge if serum or plasma is required. Use a micropipette to collect 50 μ L of the sample.

Performing the test.

Place the test cassette on a clean, level surface.

Add the sample (whole blood, serum, or plasma) into the sample well (S) of the test strip using a capillary tube or micropipette.

Add buffer solution (usually 2-3 drops) into the buffer well (B) to facilitate the reaction.

Start the timer and allow the test to run for 15-20 minutes as per the manufacturer's instructions.

Reading and interpretation of results.

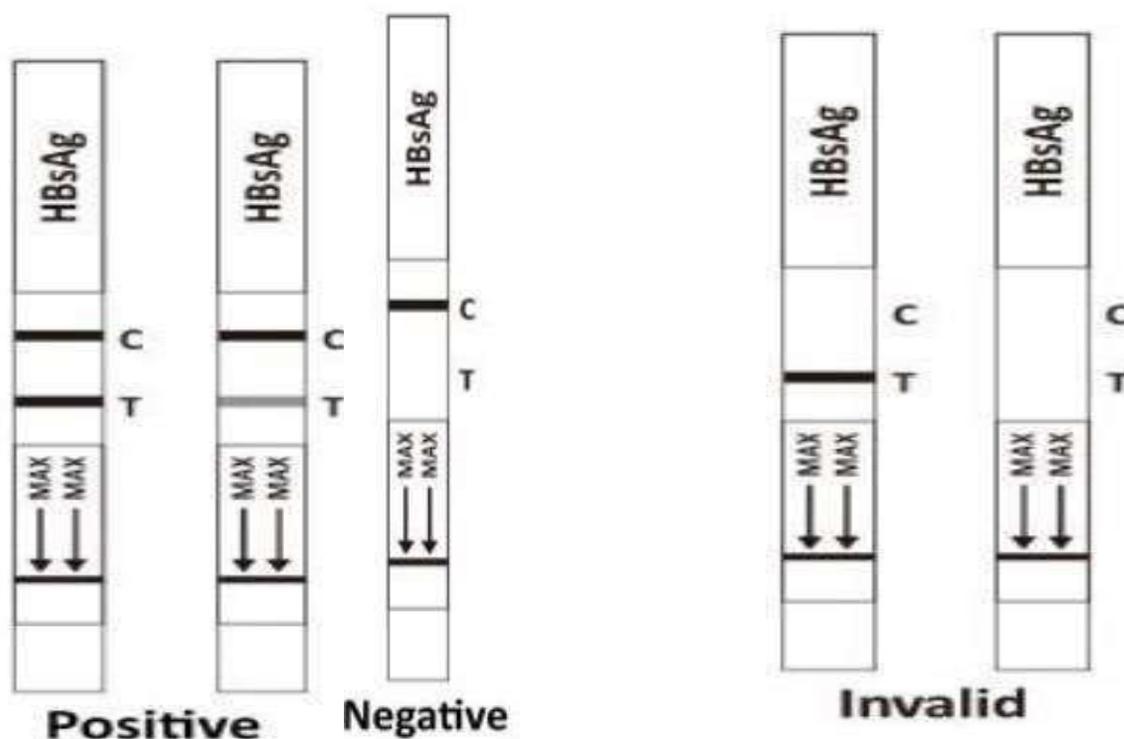
C (Control line): Indicates the test is working properly

T (Test line): Indicates the presence of hepatitis antigen.

Both (T and C lines appear): Indicates a positive test result.

Only C appears and no T line: Indicates a negative test result

Only the T line appears and no C line: Indicates an invalid test.



Quality Control and Precautions

Ensure test kits are stored at the correct temperature (usually 2–30°C).

Always use a new, sterile lancet for each patient.

Do not use expired or damaged test kits.

Read the test results within the recommended time frame to avoid false readings.

Study Variables.

Dependent Variable.

In this study, the dependent variable was the prevalence of the hepatitis B virus among pregnant mothers attending antenatal services at ERRH.

Independent Variables.

The independent variable of this study was social demographic factors and predisposing factors.

Quality Control of the Study.

The questionnaire was pretested to ensure validity and reliability.

All materials used were checked for their expiry dates.

Reference serological charts were displayed on the bench for results comparison.

The study used a parallel check by different technicians to control the results obtained to avoid transmission of infections.

Data Analysis and Presentation.

Data collected was coded, analyzed, and entered into SPSS software, whose results were used to generate tables, pie charts, and graphs.

Ethical Consideration.

The report was first approved by the Mildmay School of Medical Laboratory, after which an introductory letter was provided by the principal tutor. Permission to carry out the study was sought from the Lab manager, ERRH, after submitting the approved report and the introductory letter. The purpose and benefits of the study were explained to the participants, and whether they had the right to participate or not.

Data collection was carried out on only those who were willing to participate and consented by signing the consent form, but had the right to withdraw from the study at any time they



wished, and their decision was respected without jeopardizing their stay at ERRH. Privacy and confidentiality were observed by giving codes to respondents with numbers to avoid names.

Results. Socio-Demographic Characteristics of Respondents.

Table 1: The Socio-Demographic Characteristics of the Participants (n=128)

Variable	Category	Frequency (n)	Percentage
Age (years)	18–24	35	27.3
	25–29	45	35.2
	30–34	30	23.4
	≥35	18	14.1
	Total	128	100
Marital Status	Married	82	64.1
	Single	26	20.3
	Divorced/Separated	20	15.6
	Total	128	100
Education Level	Primary	18	14.1
	Secondary	45	35.1
	Tertiary	44	34.4
	None	21	16.4
	Total	128	100
Occupation	Housewife	38	29.7
	Business	46	35.9
	Formal Employment	26	20.3
	Other	18	14.1
	Total	128	100
Residence	Urban	86	67.2

	Rural	42	32.8
	Total	128	100

Page | 6 Most respondents were aged 25–29 years, 45 (35.2%), and married, 82(64.1%). The majority had attained at least secondary education, 45(35.1%), most resided in urban areas, 86(67.2%), and most of the correspondents were involved in the business sector, 46(35.9%), as their occupation.

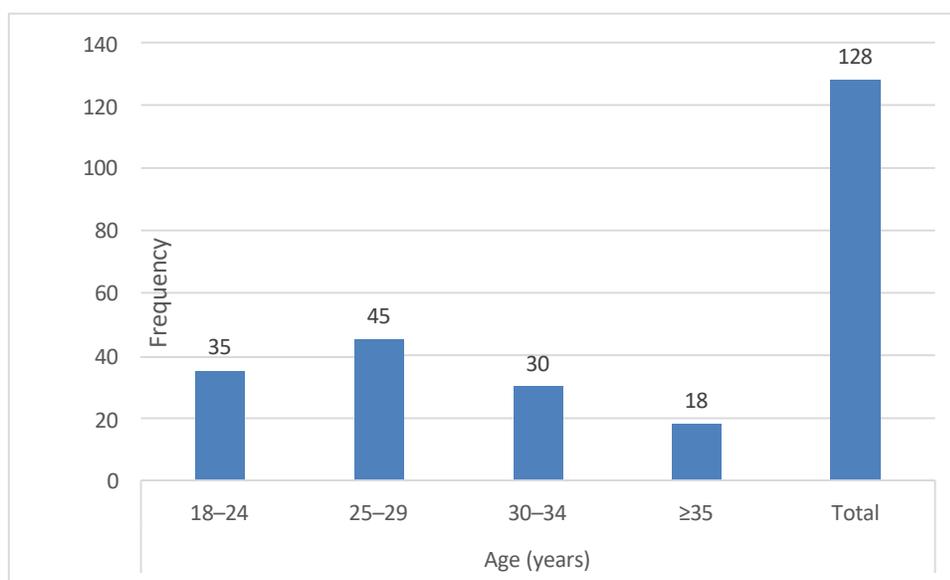
Prevalence of Hepatitis B Virus Infection.

Table 2: Prevalence of HBV infection

Variable	Category	Frequency (N=128)	Percentage (%)
Tests(status)	Positive	9	7.0
	Negative	119	93.0
	Total	128	100

Out of the 128 respondents tested for HBsAg, 9 tested positive, representing an overall prevalence of 7.0%, and 119(93.0%) tested negative.

Figure 1: Showing the distribution of HBV infection according to age.



The highest infection rate was observed among mothers aged 25-29 years (45/128), followed by those aged between 18-24 years (35/128), those aged 30-34 years (30/128), and lastly those above 35 years (18/128).

Risk Factors associated with HBV infection among pregnant mothers.

Table 3: A table showing risk factors that were analyzed in relation to HBV infection.

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Risk Factor	Category	No. Tested	Positive (n)	Prevalence (%)	Negative(n)	Prevalence (%)
History of Blood Transfusion	Yes	24	2	8.3	22	91.7
	No	104	7	6.7	97	93.3
	Total	128	9	100	119	100
Multiple Sexual Partners	Yes	28	4	14.3	24	85.7
	No	100	5	5.0	95	95
	Total	128	9	100	119	100
History of Tattooing/Piercing	Yes	36	3	8.3	33	91.7
	No	92	6	6.5	86	93.5
	Total	128	9	100	119	100
Sharing of Sharp Instruments	Yes	16	2	12.5	14	87.5
	No	112	7	6.3	105	93.7
	Total	128	9	100	119	100
HBV Vaccination History	Vaccinated	56	2	3.6	54	96.4
	Not Vaccinated	72	7	9.7	65	90.3
	Total	128	9	100	119	100

The highest infection rates were observed among mothers with multiple sexual partners, 14.3% (28/128), and a history of sharing sharp instruments, 12.5% (16/128). Those who had received HBV vaccination exhibited a lower infection rate, 3.6% (56/128), than those unvaccinated, 9.7% (72/128).

Social demographic factors associated with HBV among pregnant mothers.

Table 4: A table showing social demographic factors that were analyzed in relation to HBV infection.

Variable	Category	Frequency	Positive(n)	Prevalence (%)	Negative(n)	Prevalence
Age group	18-24	35	1	2.9	34	97.1
	25-29	45	3	6.7	42	93.3
	30-34	30	4	13.3	26	86.7
	≥35	18	1	5.6	17	94.4
	Total	128	9	100	119	100
Marital status	Married	82	1	1.3	81	98.7
	Single	26	5	19.2	21	80.8
	Divorced/ separated	20	3	15	17	85.0
	Total	128	9	100	119	100
Education level	Primary	18	2	11.1	16	88.9
	Secondary	45	1	2.2	44	97.8
	Tertiary	44	3	6.8	41	93.2
	None	21	3	14.3	18	85.7
	Total	128	9	100	119	100
Occupation	Housewife	38	3	7.9	35	92.1
	Business	46	4	8.7	42	91.3
	Formal employment	26	0	0	26	100
	Other	18	2	11.1	16	88.9
	Total	128	9	100	119	100
Residence	Urban	86	4	4.7	82	95.3
	Rural	42	5	11.9	37	88.1
	Total	128	9	100	119	100

The highest infection rates were observed among mothers aged between 30 and 34 years, 13.3% (30/128), those who had no education, 14.3% (21/128), those who were single, 19.2% (26/128), and those who lived in rural areas, 11.9% (42/128). The lowest infection rate was observed among pregnant mothers who had made it to secondary school, 2.2% (45/128), and those who had formal employment, 0% (26/100).

Discussions

Prevalence of HBV Infection.

The study revealed an HBV prevalence of **7.0%** (9/128) among pregnant mothers. According to the WHO classification, this represents an intermediate endemic level. The prevalence is comparable to previous findings in Uganda, for instance, while Nakwagala *et al.* (2015) recorded 7.1% at Mulago Hospital. The prevalence reported in this study (7.0) is slightly higher than that reported in a similar study conducted



at Mulago National Referral Hospital, which found a prevalence of 4.1% Nankya *et al.*, 2020), but lower than findings from northern Uganda, where the prevalence among pregnant women was 11.8% Okwen *et al.*, 2019). These variations may be attributed to differences in geographical locations, exposure risk, and vaccination coverage.

Social demographic factors associated with HBV among pregnant mothers.

The highest prevalence, 13.3% (30/128), occurred among mothers aged 30–34 years. Similar trends were noted by Ochola *et al.* (2021), who found that HBV infection increases with age due to prolonged exposure to risk factors. Single mothers had a higher infection rate, 19.2% (26/128), compared to married women, 1.3% (82/128), which may be attributed to increased likelihood of multiple sexual partnerships or less consistent healthcare engagement. Education level also played a role; women with only primary education, 11.1% (18/128), had higher infection rates than those with tertiary education, 6.8% (44/128), aligning with Kirbak *et al.* (2017), who linked limited education to lower HBV awareness and preventive practices.

Risk Factors associated with HBV infection among pregnant mothers.

This study found strong associations between HBV infection and behavioral/medical risk factors. Respondents with a history of blood transfusion, 8.3% (24/128), or multiple sexual partners, 14.3% (28/128), had significantly higher infection rates. These findings mirror those of Amazigo & Chime (2018), who identified unsafe transfusions and sexual transmission as major HBV routes in sub-Saharan Africa. Tattooing or piercing was another contributing factor, 8.3% (36/128), likely due to unsterilized equipment, consistent with studies by Ndako *et al.* (2019). Similarly, infection was higher, 12.5% (16/128), among those who shared sharp instruments — a practice still common in some cultural or domestic settings.

Vaccination showed a protective effect, with only 3.6% (2/128) infection among vaccinated participants compared to 9.7% (72/128) among unvaccinated mothers, supporting WHO recommendations for universal HBV vaccination of women of reproductive age.

Conclusion.

In conclusion, the study set out to achieve three specific objectives, and the findings revealed that the prevalence of hepatitis B virus infection among pregnant women attending antenatal care services at Entebbe Regional Referral Hospital

was 7.0% (9/128), indicating a moderate level of endemicity as classified by the World Health Organization.

Regarding the second objective, which examined the socio-demographic factors associated with Hepatitis B virus infection, the results showed no significant relationship between Hepatitis B infection and factors such as age with most being aged between 25–29 years 45/128(35.2%), education level with the most category being able to reach secondary school 45/128(35.1%), and marital status most of whom were married 82/128(64.1%).

With regards to the third objective, which sought to identify the associated risk factors of Hepatitis B virus infection, the study found that sharing sharp instruments 12.5% (16/128), having multiple sexual partners 14.3% (28/128), and lack of vaccination 9.7% (72/128) were the main contributors of HBV infection among pregnant mothers.

Limitations of the Study.

The sample size of 128 was not a true representation of the Wakiso district, and hence, the study may have been biased.

Material resources limited the study to self-administered questionnaires, HBsAg test, and excluded molecular assay, which could have assisted in answering deeper questions regarding the hepatitis B virus.

Some participants thought they had to be paid to participate in the study, which limited getting data on time.

Recommendations.

In light of the study findings, it is recommended that routine screening of Hepatitis B Virus infection be strengthened for all pregnant women attending antenatal care services in order to facilitate early detection, timely management, and prevention of mother - to- child- transmission.

Health education and sensitization programs should be intensified to increase awareness among expectant mothers and the general population regarding modes of transmission, preventive measures, and the importance of Hepatitis B vaccination.

The Ministry of Health, in collaboration with relevant stakeholders, should ensure continuous availability and accessibility of affordable Hepatitis B vaccines, particularly targeting women of reproductive age, to curb new infections. Furthermore, health care workers should be adequately trained and supported to adhere to strict infection prevention and control measures, including proper sterilization and safe handling of sharp instruments, to reduce the risk of nosocomial transmission of the Hepatitis B virus.



Acknowledgement.

First and foremost, I am profoundly grateful to the Almighty God for granting me the strength, perseverance, and wisdom to complete this research.

I would like to express my sincere gratitude to my supervisor, Mr. Ssekitoleko Anthony Isaiah, for his invaluable guidance, continuous support, and constructive feedback throughout every stage of this dissertation. His expertise and encouragement were instrumental in shaping the direction and outcome of my research.

My heartfelt appreciation goes to my parents, whose love, prayers, and constant support have been my greatest source of motivation. Your belief in me has been my anchor throughout this journey. I also extend my thanks to my former Dean, the School of Medical Laboratory Technology, as well as all the lecturers who contributed to my academic growth.

Special thanks to my friends and classmates for their encouragement, assistance, and for sharing in the ups and downs of this journey. Your presence made the process lighter and more meaningful.

List of abbreviations.

HBV — Hepatitis B Virus

HBsAg — Hepatitis B Surface Antigen

HIV — Human Immunodeficiency Virus

MTCT — Mother-to-Child Transmission

WHO — World Health Organization

ERRH — Entebbe Regional Referral Hospital

SPSS — Statistical Package for the Social Sciences

DNA — Deoxyribonucleic Acid

ANC — Antenatal Care

MOH — Ministry of Health

Source of funding.

The study was not funded.

Conflict of interest.

There is no conflict of interest.

Availability of data.

Data used in this study are available upon request from the corresponding author.

Authors contribution.

FN designed the study, conducted data collection, cleaned and analyzed data, and drafted the manuscript.

AS supervised all stages of the study from the conceptualization of the topic to manuscript writing and submission.

FA supervised the research process.

HN supervised the research process.

JFN supervised the research process.

Author's biography.

Faith Nambuya is a student of a diploma in Medical Laboratory Technology at Mildmay Institute of Health Sciences.

Anthony Ssekitoleko is a research supervisor at Mildmay Institute of Health Sciences.

Francisco Ssemuwemba is a research supervisor at Mildmay Institute of Health Sciences.

Hasifah Nansereko is a research supervisor at Mildmay Institute of Health Sciences.

Jane Frank Nalubega is a research supervisor at Mildmay Institute of Health Sciences.

Immaculate Prosperia Naggulu is a research supervisor at Mildmay Institute of Health Sciences.

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Student's Journal of Health Research Africa

e-ISSN: 2709-9997, p-ISSN: 3006-1059

Vol.7 No. 3 (2026): March 2026 Issue

<https://doi.org/10.51168/sjhrafrica.v7i3.2404>

Original Article

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PUBLISHER DETAILS

Student's Journal of Health Research (SJHR)

(ISSN 2709-9997) Online

(ISSN 3006-1059) Print

Category: Non-Governmental & Non-profit Organization

Email: studentsjournal2020@gmail.com

WhatsApp: +256 775 434 261

Location: Scholar's Summit Nakigalala, P. O. Box 701432,

Entebbe Uganda, East Africa

