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ON

Prevalence of Hepatitis B Virus Infection, Mother-to-Child Transmission and Associated Risk Factors among Mothers Attending Gambella Health Facilities, South-Western Ethiopia

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Ethiopia

PREVALENCE OF HEPATITIS B VIRUS INFECTION, MOTHER-TO-CHILD TRANSMISSION AND ASSOCIATED RISK FACTORS AMONG MOTHERS IN GAMBELLA HEALTH FACILITIES, SOUTH-WESTERN ETHIOPIA

A research thesis submitted to the Aklilu Lemma Institute of Health Research, Addis Ababa University, in partial fulfillment of the requirments for the degree of master of science in Tropical and infectious disease

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STATEMENT OF AUTHOR

I, the undersigned, declare that this research thesis entitled on “Prevalence of Hepatitis B Virus Infection, Mother-to-Child Transmission and Associated Risk Factors among Mothers Attending Gambella Health Facilities, South-Western Ethiopia” is my original work and hasnot been presented to any other university for the award of any academic degree, diploma or certificate and that all the sources of materials used for the thesis have been duly acknowledged.

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LIST OF ACRONYMS/ ABBREVATIONS

Hep B- Hepatitis B

HBV- Hepatitis B virus

CHB- Chronic Hepatitis B

HIV/AIDS- Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome

MTC/MTCT- Mother to Child/Mother to Child Transmission

HBsAg- Hepatitis B surface antigen

HBeAg- Heepatitis B envelope antigen

HBV DNA- Hepatitis B virus deoxyribonucleic acid

VL- viral load

Anti- HBe- Anti hepatitis B envelope

Anti-HBc- Anti hepatitis B core

Anti- HBs- Anti hepatitis B surface

RDT- Rapid Diagnostic Test

HepB-BD- Hepatitis B birth dose

HBIG- Hepatitis B immune globulin

LLD- Lower limit of detection

ANC- Antenatal Care

HC- Health Care

GTPH- Gambella Town Primary Hospital

GGH- Gambella General Hospital

NLHC- Newland Health Center

ICL- International Clinical Laboratories

ALIPB- Aklilu Lemma Institute of Pathobiology

CDC- Center for Disease Control and Prevention

WHO- World Health Organization

SSA- sub- Saharan Africa

SNNPR- Southern Nations, Nationalities, and Peoples Region

MOH- Ministry of Health

SOP- Standard Operating Procedure

SUMMARY

According to World Health Organization, about 2 billion people have been infected with hepatitis B virus. More than 254 million people live with chronic Hepatitis B virus infection worldwide. Most individuals with chronic HBV infection contracted the virus during perinatal period or early childhood. Infected perinatal mothers serve as a reservoir for the virus. Hepatitis B is a global public health problem, particularly in Ethiopia where the burden of the disease is compounded by high mother-to-child transmission (MTCT) rates. However there was no data on MTCT in Gambella. Therefore this study aimed to investigate the prevalence of hepatitis B virus, mother-to-child transmission and associated risk factors among mothers attending Expanded Program on Immunization clinics at health facilities in Gambella, South-Western Ethiopia. A cross-sectional study was conducted involving 350 mothers selected using systematic random sampling method. Data on socio-demography and associated risk factors were collected using structured questionnaire. Mothers were screened for Hepatitis B surface antigen (HBsAg) and then positive mothers were assessed for Hepatitis B envelope antigen (HBeAg) and viral load (VL). Exposed infants were tested for HBsAg and viral load at 9-15 months of age and a positive result for either were to be taken as evidence of MTCT. Data analysis was done using STATA version 20. The overall HBsAg seroprevalence among mothers was 7.4% (95% Confidence Interval: 5.1%-10.7%). History of abortion (AOR=3.5; 95% CI: 1.2-10.4; P=0.026), history of multiple sexual partner (AOR=5.5; 95% CI: 1.7-17.8; P=0.004) and having family member infected with HBV (AOR=3.7; 95% CI: 1.3-10.9; P=0.02) were factors significantly associated with seroprevalence of HBV infection. The prevalence of MTCT was 0% with 96.2% HBeAg negative CHB and maternal VL ranging between <10 and 15,000 IU/ml. Gambella has intermediate seroprevalence of HBV among mothers. Almost all infected mothers had chronic hepatitis B with negative HBeAg and low viral load predominance. Therefore, HBV screening should be strengthened. Mothers sould be encouraged to receive the hepatitis B. Safe and comprehensive abortion care should be provided to all mothers in need. Provision of health education about HBV and its preventive methods is inevitable. Further molecular characterization should be conducted.

Key words- hepatitis B, mother, prevalence, mother to child transmission, Risk factor, Gambella

1. INTRODUCTION

1.1 Background of the Study

Hepititis B virus (HBV) infection is caused by hepatitis B virus which is enveloped DNA virus. HBV is a hepatotropic and extremely infectious as compared with Human Immunodeficiency Virus (HIV), which is 100 times infectious. It can be transmitted either by horizontal or vertical route of transmission. Vertical transmission is the main route of transmission and major source of chronic hepatitis B infection especially in endemic countries. The importance of perinatal transmission is their remarkably greater risk of chronicity compared to infections acquired later in life (Mast *et al*., 2005).

World Health Organization (WHO) estimated about 2 billion people have been infected with Hepatitis B virus. 254 million people are living with chronic hepatitis B (CHB) and reported 1.2 million new cases annually. Hepatitis B caused 1.1 million deaths, primarily due to cirrhosis and hepatocellular carcinoma. Majority of HBV related deaths are the result of infections contracted at birth or during early childhood. The burden of hepatitis B varies widely. The highest burden observed in WHO western pacific and African region, with 97 million and 65 million chronically infected individuals. The pooled prevalence of HBV in Ethiopia is 7.4% (Assefa *et al*., 2024) (WHO, 2024a).

Mother to child transmission (MTCT) is the main route of HBV transmission, contributing significantly to the persistence of the virus in the population. The risk of developing chronic hepatitis B infection after perinatal transmission is 95% as compared to only 5% risk of infection during adulthood. A study done in West Africa, vertical transmission was linked to fivefold increased risk in developing advanced liver disease as compared to those who acquired via horizontal transmission later in life (WHO, 2022, Shimakawa *et al*., 2016).

According to Yao *et al*, the pooled MTCT incidence of HBV was 31.3% worldwide. The estimated MTCT with no intervention was 82.9% in HBeAg positive mothers. The rate decreased to 15.9% and 9.6% with administration of hepB-BD and combined immunoprophylaxis respectively. In contrast, MTCT rate among HBeAg negative mothers was 10.3% (95%CI:0.5-28.2%). With administration of hep B vaccine and comined immunoprophylaxis, the MTCT incidence decreased to 2.3% and 0.5% respectively (Yao *et al*., 2022).

The overall rate of MTCT in Ethiopia was 7.1%. In the presence of HBeAg with no immunoprophylaxis, the rate of MTCT was 57.1% and 15.8% with hepB-BD (with/without HBIG). Whereas in the absence of both HBeAg and any intervention, the rate of MTCT was 16.7% which then decreased significantly to 0.9% with hepB-BD (with/without HBIG) (Arefaine *et al*., 2024).

MTCT of hepatitis B is influenced by several risk factors, with maternal viral load (VL) being the most significant. High maternal HBV DNA level (>200,000IU/ml) increase the likelihood of transmission, even leading to immunoprophylaxis failure. Other risk factors include the presence of HBeAg, and failure to administer timely hepB-BD and HBIG to exposed neonates. Additionally pregnancy related complications including invasive procedures during pregnancy or deliver could elevate the risk by increasing fetal exposure to maternal blood (Terrault *et al*., 2018).

The presence of HBsAg in women of reproductive age or pregnant women serve as an important indicator for the potential risk of MTCT of hepatitis B virus. The pooled prevalence of HBV among pregnant women in Africa was 6.77%. In sub-Saharan Africa, the seroprevalence of HBsAg in pregnant women remained high (up to 20%). In Ethiopia, the pooled prevalence of HBV was 5.78% (Duri *et al*., 2023; Kabore, 2023; Asgedom *et al*., 2024; Larebo *et al*., 2024).

WHO identified prevention of MTCT of hepatitis B as a key priority in reaching the elimination goal of 2030, particularly due to residual risk of MTCT among children born from high risk mothers. Intervention measures include routine prenatal HBsAg screening for pregnant women; providing antiviral drugs at 28th week of gestation to pregnant women with high viral load; ensuring timely hep B-BD& HBIG to exposed neonates and conducting routine follow up for the exposed infants (WHO, 2021; Shimakawa *et al*., 2022).

According to CDC, infants born from hepatitis B positive mothers should be tested after completion of hepatitis B vaccine series, typically between 9 and 12 months of age to confirm the infant's infection status and to determine effectiveness of the vaccine. Screening is not recommended before age of 9 month to avoid detection of passive antigenemia (CDC, 2024).

In Ethiopia, limited studies have been conducted on the prevalence of HBV, MTCT rates, and associated risk factors among mothers. Gambella, being one of the regions with unique demographic and health challenges, is likely to bear a considerable burden of HBV. Even though

pregnant women are screened and found to be positive, there is no well-organized care pathway to initiate eligible mothers on antiviral therapy and further provide immunoprophylaxis to their newborns. In addition breastfeeding in the absence of immunoprophylaxis could increase the risk of postnatal transmission. Sociocultural, behavioral, medical and obstetric factors and lack of appropriate preventive measures could further exacerbate the situation. Understanding the epidemiology of HBV and MTCT in this context is crucial to design targeted interventions. The findings of this study could be used to design appropriate prevention and control measures against HBV infection.

1.2 Statement of the Problem

Hepatitis B virus (HBV) infection remains a significant global health concern, particularly in regions with high endemicity such as sub-Saharan Africa. Despite being entirely vaccine preventable, the burden of chronic HBV continues to rise, leading to severe health complications including liver cirrhosis, hepatocellular carcinoma, and increased mortality rates. Perinatal mothers are particularly high risk group due to MTCT risk, which maintain HBV infection across generations (Johannessen *et al*., 2021).

In Ethiopia, and specifically in the Gambella region, the prevalence of HBV among pregnant women was evident. Nevertheless the scope was narrow and no study was done on MTCT. Preliminary data suggest that the rate of infection in this area is higher than the national average due to factors such as increasing prevalence (6%) of HIV/AIDS, polygamy which is widely accepted and practiced, and "Gaar" which is scarification of male's forehead. Other potential factors include limited healthcare access, insufficient vaccination coverage, and socio-cultural practices. This poses a significant public health challenge and vertical transmission from mother to child should be averted with appropriate interventions, including comprehensive prenatal screening programs and timely administration of hep B-BD (Kibret *et al*., 2019; Tanga *et al*., 2019; Ayele *et al.*, 2020).

Understanding the extent of the problem and identifying the key determinants are critical steps toward developing effective public health strategies and intervention programs. Addressing this gap is essential to reduce the transmission of HBV and improve maternal and neonatal health outcomes in the region.

1.3 Significance of the Study

Therefore, this study aimed to investigate the prevalence of HBV infection, rate of mother to child transmission and identify significant associated factors among mothers attending Expanded Program on Immunization (EPI) to get their children vaccinated in Gambella. The findings will provide valuable insights for healthcare providers and policymakers including Gambella Regional Health Bureau to design and implement targeted interventions to curb the spread of HBV in this vulnerable population. The study can further be used as baseline information for future study

2. OBJECTIVES

2.1 General objective

This study aimed to estimate the prevalence of hepatitis B virus infection, mother to child transmission, and its associated risk factors among mothers attending EPI clinic in Gambella Health facilities, South Western Ethiopia.

2.2 Specific objectives

- To estimate the seroprevalence of HBsAg in mothers attending EPI clinic in Gambella
- To determine the prevalence of mother to child transmission in Gambella
- To identify associated risk factors with hepatitis B infection and MTCT among mothers attending EPI in Gambella

3. LITERATURE REVIEW

3.1 Overview of hepatitis B

Hepatitis B is under Hepadnaviridae family which is double stranded enveloped DNA virus measuring 3.2 kbp in its genome. Its genome is arranged distinctly where the minus strand of the DNA carry gene encoding for both structural protein (pre-s, surface, and core) and replicative proteins (polymerase and x protein) forming complete circle. On the other hand the plus strand of the DNA is shorter and vary in length. Certain viral proteins such as HBcAg, HBeAg, DNA polymerase are found in the inner core of the virus. HBsAg is found on the surface of the virus. The virus primarily affect the liver cell, hepatocytes where it undergoes replication (Chuang *et al*., 2022).

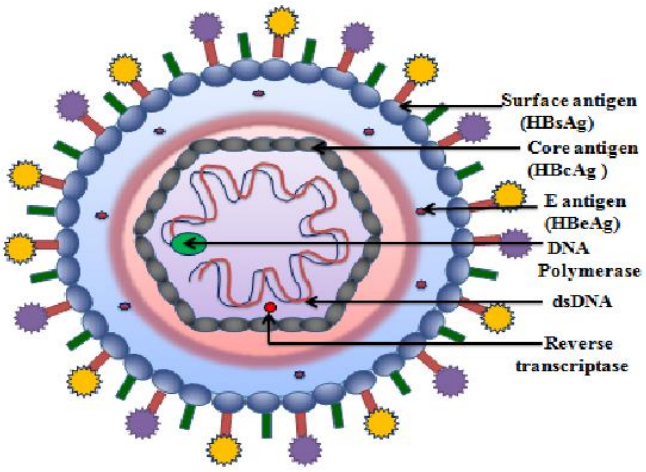


Figure 1. Structure of Hepatitis B

Source- (Singh and Sinha, 2015)

Electron microscopy of infectious serum reveals three types of viral particles. Two of these are noninfectious and include smaller spherical structures approximately 20 nm in diameter, as well as filaments with variable lengths and a consistent width of 22 nm. Both types consist of HBsAg and host-derived lipids but lack viral nucleic acids. In contrast, the infectious HBV virion, known as the Dane particle, is a spherical, double-shelled structure measuring 42 nm in diameter. It features a lipid envelope containing HBsAg, which encloses an inner nucleocapsid composed of hepatitis B core antigen (HBcAg), a virally encoded polymerase, and the viral DNA genome. HBeAg is a small soluble secretory protein produced by HBV (Ott *et al*., 2012; Hu and Liu, 2017).

3.2 Clinical course and serological changes during HBV infection

The average incubation period of HBV is 75 days, can range 1-3 months. Hepatitis B can cause wide spectrum of liver disease ranging from acute to chronic hepatitis. The virus can persist into chronic hepatitis B especially in patient who acquired the infection during infancy or early childhood. Acute hepatitis B progresses with the early detection of HBV DNA, HBsAg, and HBeAg in serum. HBsAg appears 1–12 weeks after exposure and its persistence indicates chronic infection. HBeAg signifies high viral replication and infectivity. Rise in liver enzyme levels and jaundice often develop within weeks of viral marker appearance. HBeAg is typically cleared at the peak of illness with antibodies (anti-HBc, anti-HBe, and anti-HBs) arising in distinct patterns. Anti-HBc IgM appears first, followed by IgG. Anti-HBe follows with HBeAg clearance, signaling recovery. Then anti-HBs develops later, conferring immunity. However, 10–15% of recovered patients lack detectable anti-HBs, making anti-HBc better indicator of past infection. Chronic hepatitis have persistent viral replication and high titers of HBsAg, HBeAg, and HBV DNA, often resulting in long-term liver damage, cirrhosis, and a heightened risk of hepatocellular carcinoma (HCC) (Liang, 2009; WHO, 2016).

Various studies have stated controversial hypothesis on immune response of infants to perinatal HBV infection. The theory of immature and/ or immunotolerance phase are being challenged due to expression of HBV specific T-cell response as well as HBV core and polymerase specific T-cell response after immunoprophylaxis treatment. However, the weak proinflammatory immune response lead infected infants to chronic hepatitis carriers (Komatsu *et al.*, 2010; Bertoletti and Hong, 2014).

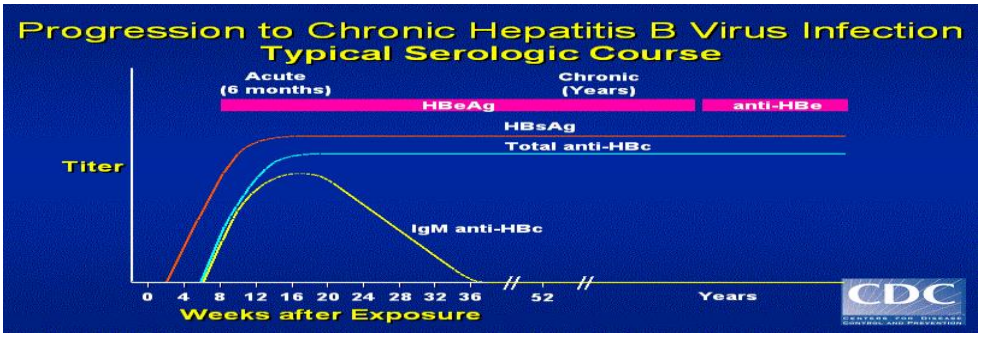


Figure 2 – clinical and serological course of hepatitis B infection

Source- https://www.microbiologybook.org/virol/hepb-cd2.gif

3.3 Hepatitis B serological markers in infants

Studies showed the potential of maternal HBV markers to be passed to the fetus through the placental barriers. While in majority of cases placental barriers effectively prevents the transmission of HBsAg, HBeAg and HBV-DNA, it facilitates the transfer of antibodies (anti-HBe and anti-HBc) from mother to fetus via active transport of IgG antibodies. In high risk infants, HBV markers present at birth often disappeared by 8-12 months, suggesting that presence of such markers at birth do not necessarily reflect in-utero infection. Serologic markers that are most likely to be lost first are HBeAg and HBsAg followed by HBV-DNA, anti-HBe and anti-HBc. Follow up data revealed that HBV markers such as HBeAg, HBsAg, and HBV-DNA have loss rates of 88.7%, 88.1% and 64.3% respectively by 8-12 months. Conversely, HBV markers absent at birth were occasionally detected later suggesting possible infection during labor and delivery; breast feeding in the absence of immunoprophylaxis or early horizontal transmission. These results challenge previous assumption that HBV markers at birth are definitive for in-utero infection.

This underlines "positivity of HBV markers at birth cannot universally be used to define in-utero infection and negativity for HBV markers cannot be used to exclude maternal-infant transmission". Therefore, to completely understand maternal-infant transmission, comprehensive follow-up is essential, particularly at 8-12 months when both infection and immunity markers are most reliably detectable. These findings emphasize the need for continuous follow up of high-risk infants to clarify the timing and mechanisms of HBV transmission (Zhang *et al*., 2016).

3.4 Role of HBV proteins in MTCT

Maternal *HBsAg* level are positively associated with the risk of mother to child transmission. *HBeAg* plays an immunomodulatory role in virus-host interactions, promoting immune tolerance in neonates exposed in utero. Maternal HBeAg transfer renders infants' T helper cells unresponsive to HBeAg and HBcAg, leading to persistent HBV infection and chronicity in 90% of cases. In contrast, transmission from HBeAg negative mothers often triggers immune clearance, causing acute or fulminant hepatitis but rarely chronic infection (5%). Both maternal HBeAg status and serum *HBV-DNA* level are critical indicators of viral replication. In pregnant women with viral loads of >3 log10 copies/mL, *functional hepatitis B X protein* (HBx) produced in HBV-infected placenta cells could activate phosphoinositide 3-kinase in placenta, which signals inhibition of

apoptosis in placental cells, allowing for HBV persistence in trophoblasts (Chang, 2007; Bai *et al*., 2012).

3.5 HBV screening

According to WHO, all countries should integrate HBsAg screening service to identify pregnant women who are infected with HBV. Viral markers such as HBsAg, HBeAg, Hepatitis B surface antibody (anti-HBs) and Hepatitis B core antibody (anti-HBc) are commonly used to diagnose hepatitis B infection. HBsAg with HBeAg and Viral DNA are used for screening, assessing risk of MTCT, treatment initiation (antiviral prophylaxis) and infant immunoprophylaxis (WHO, 2017).

3.6 Epidemiology

3.6.1 Mode of transmission

Hepatitis B is extremely infectious, as compared with HIV and Hepatitis C, it's 100 times and 10 times infectious respectively. Hepatitis B transmission are broadly categorized as horizontal and vertical transmission. Horizontal transmission is when HBV is transmitted from person to person via percutaneous and mucous membrane contact with blood and/or contaminated body fluid including saliva, vaginal fluids, and seminal fluids of infected individual. Use of contaminated traditional or medical equipment, blood donation, sexual contact are among horizontal mode of transmission. Practices such as tattooing, piercing, use of injectable drugs increase the risk of infection. Vertical transmission is common route of transmission from mother to child. Mother to child transmission can occur during pregnancy, birth or early horizontal transmission. Early horizontal transmission is through exposure to contaminated blood or body fluid commonly from the mother or infected child till age 5 years (WHO, 2024a; Bafa and Egata, 2020).

3.6.2 Global distribution of Hepatitis B

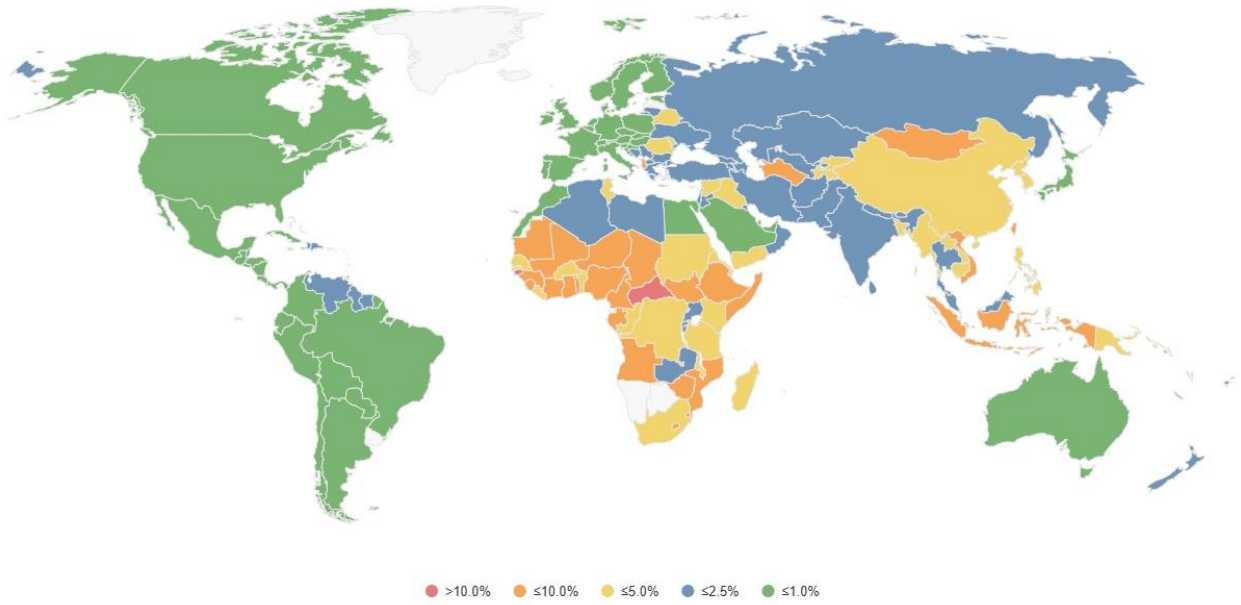


Figure 3. Hepatitis B surface Antigen prevalence of 2024

Source- https://cdafound.org/polaris-countries-distribution/

Though slight decrement is observed in subsequent years, hepatitis B still poses significant public health challenge. In 2019, globally about 1.5 million people were newly infected with hepatitis B and reported 296 million CHB cases with 820,000 deaths from CHB related complications despite the advance in vaccine coverage. In the western pacific region, the overall prevalence of hepatitis B in the general population was 5.92%. The incidence of hepatitis B and estimated death due to hepatitis B was 140,000 and 470,000 respectively. Where as the incidence of hepatitis B and estimated death in Africa region was 990,000 and 80,000 respectively. Sub-Saharan Africa was responsible for 66% of all new CHB infection. The major (99%) cause of death was cirrhosis and liver cancer (WHO, 2021).

Hepatitis B virus infection is a major health problem in Ethiopia. Ethiopia has intermediate to high level of endemicity. The pooled prevalence of HBV in Ethiopia was 6%. Reports showed significant variation in HBV prevalence among regions. Among refugees in Gambella region, the prevalence of HBV was estimated to be 7.3% (Yazie and Tebeje, 2019; Ayele *et al.*, 2020).

Epidemiological research indicates the age distribution of HBV infection differs by region, with approximately 1/3rd of chronic cases being contracted during infancy and early childhood (Mackie *et al*., 2009).

3.6.3 Geographic pattern of Hepatitis B

Based on level of endemicity, countries can be categorized under low, intermediate or high HBV endemicity depending on the prevalence of HBsAg in the general population of a specific geographic location. High HBV endemicity is when the prevalence of HBsAg ≥8%. Intermediate HBV endemicity is when the prevalence of HBsAg ranges between 1-7%. Low HBV endemicity is when the prevalence of HBsAg <1% (Hwang and Cheung, 2011).

Around 45% of the global population, those residing in parts of Africa, Asia, the Amazon Basin and parts of the Middle East are considered to live in regions of high endemicity with lifetime risk of infection exceeding 60%. The most common route of transmission is vertical transmission or early childhood infection. Though it is difficult to determine the exact disease burden of hepatitis B in Africa due to underestimation and imprecise report, 70-90% of the adult population is estimated to have evidence of past exposure to HBV infection. The estimated HBsAg seroprevalence ranges from 6-20%. Areas of intermediate endemicity include Eastern and Southern Europe, Russia, Central & South America. The lifetime risk of infection vary between 20 and 60%. Countries such as United States, Western Europe and Austria accounting 12% of world's population, are areas of low endemicity with less than 20% lifetime risk of infection. The common mode of transmission is horizontal transmission (Hwang and Cheung, 2011).

3.6.4 Prevalence of hepatitis B in pregnant women

The prevalence of hepatitis B in pregnant women is proportionate to the general population of the region. Currently, high prevalence of HBV among pregnant women remains in African countries, whereas the prevalence of HBsAg-positive pregnant women in Europe and America is low. China had substantial reduction in the proportion of hepatitis B among pregnant women. Belopolskaya review reported the highest prevalence in Republic of South Sudan and least in United states *(*Belopolskaya *et al*., 2021).

A review showed the overall prevalence of HBV infection among pregnant women in Africa was estimated to be 5.89% (95% CI: 5.26-6.51%). Among the different regions of Africa, West Africa

(6.98%) and Central Africa (6.77%) had the highest prevalence followed by East Africa (5.38%). Southern and Northern Africa reported lower prevalence rates of 1.79% and 3.75% respectively. At country level, the prevalence ranged widely between 0.74% in South Africa to 25.7% in Angola (Wondmeneh and Mekonnen, 2024).

In Ethiopia, the pooled prevalence of HBV infection among pregnant women was 4.75%, with the highest (7.9%) in Gambella and the lowest in Southern Nations, Nationalities, and Peoples' Region (SNNPR) (2.3%). In recent review, seroprevalence increased to 5.78% with the lowest reported in Addis Ababa and highest in Somalia (Alemu *et al*., 2020; Asgedom *et al*., 2024). Recent reports on the prevalence of HBV among pregnant women summarized in (Table 1).

3.6.5 Mother to Child Transmission of HBV

3.6.5.1 Mechanism of MTCT

Generally the possible routes of mother to child transmission could be categorized as prenatal, natal and postnatal transmission. Prenatal transmission is when HBV is transferred to the fetus in utero and is the leading cause of immunoprophylaxis failure. Natal transmission occurs at the time of labor and delivery when the fetus is exposed to infected maternal blood through tear of placenta during contraction or swallowing of maternal cervical secretion/ blood while passing through birth canal. Presence of HBV in breast milk and close contact with infected maternal blood could expose the infant to HBV infection, namely postnatal transmission. The transmission rate during prenatal and natal period accounted 3-8% and 35% respectively (Navabakhsh *et al*., 2011; Joshi and Coffin, 2020).

Study on the path of prenatal (intrauterine infection) is still ongoing but stated hypothesis includes

A breach in the placental barrier leading to trans-placental leakage introduces HBV infected blood (HBeAg positive maternal blood) to the fetus. Risk factors such as threatened preterm labor, abortion, trans placental infection and certain procedures such as amniocentesis increase the risk of transmission. Ascending infection from HBV infected vaginal secretion is other possible way of intrauterine infection. Studies have shown that infection could also occur at the time of conception as HBV-DNA is present in the oocytes or sperm of infected females and males respectively (Zhang *et al*., 2004).

Researches have different conclusion on the effect of mode of delivery. According to WANG *et al* report, with standard passive-active immunoprophylaxis, there were no significant difference on transmission rate among different delivery mode. A study provided evidence on the effective reduction of MTCT by elective caesarean section over vaginal delivery (WANG *et al*., 2002; Peng *et al.*, 2018).

Studies confirmed the presence of HBV in breast milk especially in colostrum and could be route for MTCT after delivery. However with the provision of standard hepatitis B immunoprophylaxis, there is no additional risk of infection among breast fed infants in positivity rate and immunoprophylaxis failure rate as compared to bottle fed infants (Wang *et al*., 2003).

3.6.5.2 Rate of MTCT

Based on a systemic review, the incidence of HBV MTCT was 31.3% with the lowest rate seen in European region and the highest in western Pacific region. MTCT incidence in South East Asia and Africa was 15.3% and 13.7% respectively. There was significant decrement with introduction of hepatitis B vaccine and combined immunoprophylaxis in south East Asia (Yao *et al*., 2022).

Globally the rate of MTCT of HBV varies depending the maternal infective status and the presence of preventive measures. Without intervention, the risk of mother to child transmission is 70-90% when mothers are positive for both HBsAg and HBeAg. In contrast the transmission risk decrease to 10-40% if mothers are only positive for HBsAg (Schillie, 2017).

The rate of MTCT in the South East Asia and Western Pacific region were 0.0-5.2% in infants born to mothers who were HBsAg+ve/ HBeAg-ve. Whereas the rate (2.7-53.0%) increased in infants born to mothers who were positive for both HBsAg and HBeAg (Marjenberg *et al*., 2022). In China, the rate of MTCT was 5.21%. HBV infected infants born from HBeAg positive mothers was 84.6% and those born from HBeAg negative mothers was 15.4%. Interestingly, HBeAg negative mothers who transmitted the virus had significantly lower maternal VL and higher HBsAg level than HBeAg positive mothers. When both HBV DNA >2,000IU/ml &HBsAg>100,000IU/ml in HBeAg negative mothers, the risk of MTCT increased with risk ratio of 26.062 (2.633-258.024) (Chen *et al*., 2018).

A review found that the average rate of mother-to-child transmission (MTCT) of hepatitis B in Sub-Saharan Africa was 38.3%, which is lower than reports from highly prevalent countries such

as Asia. Among HBeAg-negative mothers without any preventive measures, the transmission risk was 4.8%, close to the commonly stated 5–40% range. In infants born to HBeAg-negative mothers, both delayed vaccination (after the first week) and timely birth doses resulted in zero transmission (0.0%), significantly lower than unprotected cases. Surprisingly, MTCT in infants born to HBeAg-positive mothers who received the hep B-BD either timely (36.6%) or late (32.4%) showed no significant reduction in transmission as compared to those who received no intervention (Keane *et al*., 2016). The finding was quite intriguing as the result contradict most studies regarding the effectiveness of hepB-BD in infants born to HBeAg positive mothers.

In Ethiopia, 17.6% of HBsAg positive pregnant women were high risk (HBeAg positive and HBV DNA>200,000IU/ml). The overall rate of MTCT is 7.1%. Among infants who received no immunoprophylaxis, MTCT rate was 24.2%. With hep B-BD and combined (hepB-BD and HBIG) were 8.7% and 1.8% respectively. High maternal VL and no immunoprophylaxis was significantly associated with MTCT of HBV (Arefaine *et al*., 2024).

Review done by Taye *et al*, (2023) reported pooled risk of MTCT of 25.5% and 20.7% mong HIV negative women. But Tang and Zhao critically reviewed and questioned the report stating the analysis was overestimated. The reasons for that was the inclusion of article before the introduction of hepatitis B vaccine and also considering the presence of HBsAg in cord blood as HBV infection rather than exposure (Tang and Zhao, 2024).

3.6.5.3 Prevalence of HBsAg in Children

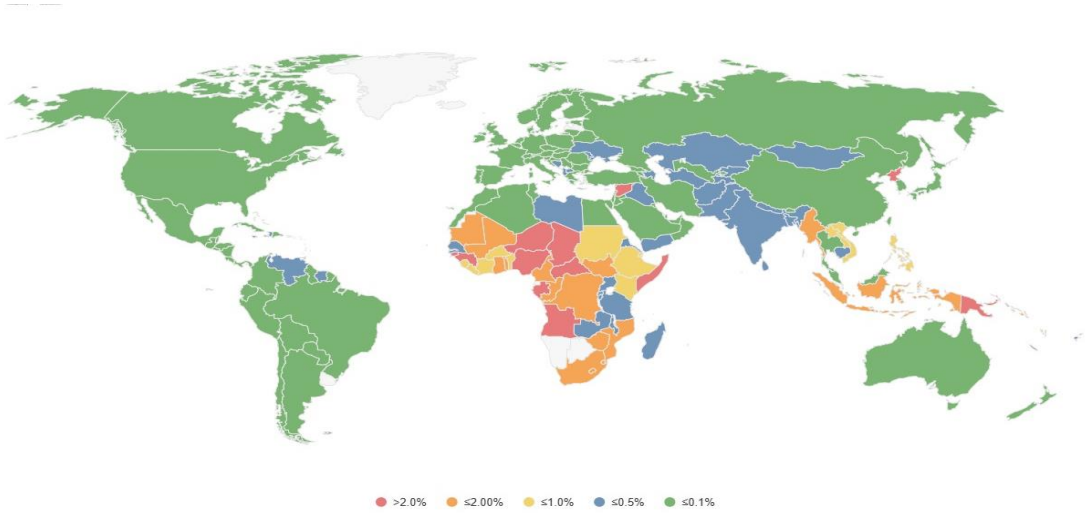


Figure 4. HBsAg prevalence among <5 yrs old children

Source- https://cdafound.org/polaris-countries-distribution/

Among children under 5yrs of Western Pacific region, the prevalence was notably lower (0.46%). Out of 37 countries, 21 of them have met the 2030 goal of reaching <1% HBsAg prevalence among children under 5yrs (WHO, 2021).

In China, there was a significant decline in HBsAg prevalence among children (10.4% in 1992 to 0.8% in 2014). This dramatic reduction is closely linked to the expansion of infant vaccination programs, including timely birth dose administration, which has seen increasing coverage rates over time. The integration of the hepatitis B vaccine into EPI further supported this success, demonstrating both sustained vaccine coverage and a notable reduction in chronic HBV cases among preschool children, evidenced in Thailand. These outcomes underscore the critical role of widespread and timely HBV vaccination in reaching the WHO's goal of eliminating hepatitis B as a public health threat by 2030 (Marjenberg *et al*., 2022).

The seroprevalence of HBsAg among children in African region remains high, accounting for more than two third (4.3 million, approximately 69%) of all infected children worldwide and lack far beyond WHO 2030 target (CDC, 2024).

3.6.5.4 Risk Factors Associated with HBV infection

HIV/HBV co-infection could result in severe progression of hepatitis B due to increase in viral replication and reactivation rates of HBV. An estimated 2.6 million HIV/HBV co-infected people are found in SSA. HIV/HBV co-infection increase the potential risk of perinatal transmission and also advancement of chronic hepatitis infection. A study done in West Africa showed 2.5 times increased risk of MTC transmission of HBV in pregnant women co-infected with HIV/HBV (Sangaré *et al*., 2009; Kourtis *et al.*, 2012).

Pregnant women co-infected with HIV and HBV are *twice* as likely to test positive for HBeAg, *three times* higher serum concentrations of HBV DNA compared to those infected with HBV alone. This significantly increase the risk of MTCT (Hoffmann and Thio, 2007).

Generally socio-demographic, social, behavioral, medical and obstetric factors could increase risk of exposure to HBV infection. Factors such as history of abortion, history of blood transfusion, hospitalization, history of surgical procedure, tattooing and history of tooth extraction were found to be significantly associated with HBV infection (Asgedom *et al*., 2024). They vary depending on the geographic location and cultural practices. The following table (Table 1) summarizes prevalence and significant risk factors associated with HBV infection among pregnant women in Ethiopia. It was also used to prepare questionnaire in the study.

Table 1. Prevalence of HBsAg and significant risk factors among pregnant women in Ethiopia

No.	Study Area	Sample size	Study Site	Prevalence of HBsAg (%)	Associated risk factor	Reference
1.	Eastern Tigray	385	General hospital	10.4	Low educational level, history of (ear piercing, abortion, home delivery, genital mutilation), being unmarried	(Abay *et al*., 2024)
2.	North-West Ethiopia	338	Referral hospital	8.3	Tattooing, multiple sexual partners, family history of HBV	(Demeke *et al*., 2021)

3.	Hararghe	302	Public hospital	8	Tattooing, multiple sexual partners, history of (tonsillectomy, contact with jaundiced person)	(Umer *et al*., 2023)
4.	Gambella	253	Hospital	7.9	History of abortion, occupation, multiple sexual partners	(Tanga *et al*., 2019)
5.	Wolaita, Southern Ethiopia	675	Public Hospital	7.3	History of multiple sexual partners, surgical procedure, genital mutilation, tooth extraction	(Bancha *et al*., 2020)
6.	Yirgalem	475	Referral hospital	7.2	History of multiple sexual partner, HIV	(Amsalu *et al*., 2018)
7.	Eastern Ethiopia	318	Referral hospital	6.9	History of abortion, nose piercing, surgical procedure, multiple sexual partners	(Umare *et al*., 2016)
8.	West Hararghe	363	Public hospital	6.1	History of (abortion, tonsillectomy, admission to heath facility), multiple sexual partners, familial liver disease	*(Mamuye et al*., 2020)
9.	Amhara	1121	Referral hospital	4.6	History of multiple sexual partners, blood transfusion, tattooing, HIV, family history of HBV	(Dagnew *et al*., 2020)
10.	Addis Ababa	12,318	Public hospital	3.04	Tattooing, multiple sexual partners, family history of HBV, sharing sharp objects	(Tesfu *et al*., 2023)

3.6.5.5 Risk factors associated with MTCT

The risk of mother to child transmission of HBV is closely related to maternal viral replication levels and infective status. Higher maternal HBV-DNA level and HBeAg positivity significantly increase transmission risk, with HBV-DNA level being a stronger predictor of transmission risk. Infants born of highly viremic mothers (> 8 log copies/ml) face 9-39% chance of vertical transmission, even with immunization. Report showed the strong correlation of MTCT with HBeAg and high viral load, MTCT was 3% in HBeAg positive and high VL. But 0% among

HBeAg negative and low VL. Maternal HBV DNA level of ≥200,000 IU/ml is considered the critical threshold for risk of MTCT (Wiseman *et al.*, 2009; Boucheron *et al*., 2021).

The prevalence of HBeAg among women of reproductive age group ranged between 20-50%. East Asia reported the highest prevalence among young females, reaching 78%. While lower rates were observed in sub-Saharan Africa and North Africa in 1999. By 2005, significant reduction was reported in some areas yet the highest prevalence was still reported from south-east Asia and the lowest prevalence from southern sub-Saharan Africa (Ott *et al*., 2012).

A study stated the seroprevalence of HBeAg in HBsAg positive was 23%. But less than 1% of women of childbearing age were positive for HBeAg in Addis Ababa. However, MTCT remained high in the region (13.7%). In addition, the diagnosis of CHB at young age indicate the acquisition of HBV infection perinatally. This is mainly due to much lower chance of HBeAg and HBsAg clearance as compared to horizontal transmission (Abebe *et al*., 2003; Nayagam *et al*., 2020; Yao *et al*., 2022).

The risk of MTCT can be categorized into three groups based on the major determinants of vertical transmission, HBeAg and HBV viral load. High risk of vertical transmission is when HBsAg is positive *And* positive HBeAg or high viremia, Low risk is when HBsAg is positive *And* negative HBeAg or low viremia. Whereas No risk is when HBsAg is negative. The prevalence of HBeAg among women of childbearing age in African region is lower than those in south East Asia (WHO, 2021; Ansari *et al*., 2023).

HBV genotypes (A-H) vary in region, influencing maternal viral load and MTCT rates. Genotypes B and C are dominant in Asia, where MTCT rates are higher due to positive HBeAg and high viral loads. In Mediterranean and neighbouring countries, genotype D is the dominant (>90%), where HBeAg negative CHB predominate. In contrast, genotypes A and E are prevalent in sub-Saharan Africa, where women often seroconvert to anti-HBe before childbearing age, reducing MTCT risk. In Ethiopia, the dominant HBV genotype are A followed by genotype D with A1 and D1 subgenotypes. Less commonly found genotypes are C, E and G. Subgenotype A1 which is commonly found in Africa, even in Ethiopia, has more hepatocarcinogenetic nature resulting in HBV related HCC at younger age (Sinha and Kumar, 2010; Hadziyannis, 2011; Assefa *et al*., 2024).

Majority of HBV infection in unvaccinated infants occur perinatally. HBV maternal-infant transmission still occur after passive-active immunization which has been proven to protect more than 90% against HBV infection. Intrauterine transmission is a major factor for immunoprophylaxis failure. Other factors include having high maternal VL and positive HBeAg. Around 9% of infants suffer from MTCT particularly in mothers with high viremia *(Kang et al*., 2014; Yi *et al.*, 2016).

Pregnancy related complication could increase the risk of MTCT. It includes preterm rupture of membrane, preterm labor, prolonged labor, invasive procedure or failure of immunoprophylaxis in previous delivery (Pan *et al*., 2012).

3.6.5.6 Prevention and Control

WHO has aimed to eliminate hepatitis B from major public health problem by 2030 by reducing MTCT of HBV to 50% and less than 0.1% HBsAg in children. The interventions planned include scaling up coverage of infant vaccination to a minimum 90% of infants, birth dose vaccination to a minimum 90% of neonates within 24 h of birth (WHO, 2016).

WHO recommended administering antiviral prophylaxis at 28^{th} week of gestation to HBsAg positive women with high viral load (≥200,000IU/ml) or HBeAg positive, alongside timely hepB-BD followed by universal hepatitis B vaccination. The combined administration of hepatitis B immune globulin (HBIG) and the HepB-BD to newborns within 24 hours of birth prevents mother-to-child transmission (MTCT) of HBV in 80–95% of cases. Provided standard immunoprophylaxis and outweighing the benefit over risk, breastfeeding is not contraindicated for infants born form hepatitis B infection. But precautions needed if mother's nipples are cracked or bleeding (Lee *et al*., 2006; WHO, 2020; CDC, 2024).

Preventing mother-to-child transmission (MTCT) of HBV is a clinically effective and practical public health strategy proved to be cost-efficient, significantly lowering the HBV burden, reducing hospitalizations, and decreasing mortality associated with advanced liver diseases caused by HBV (Taye *et al*., 2023).

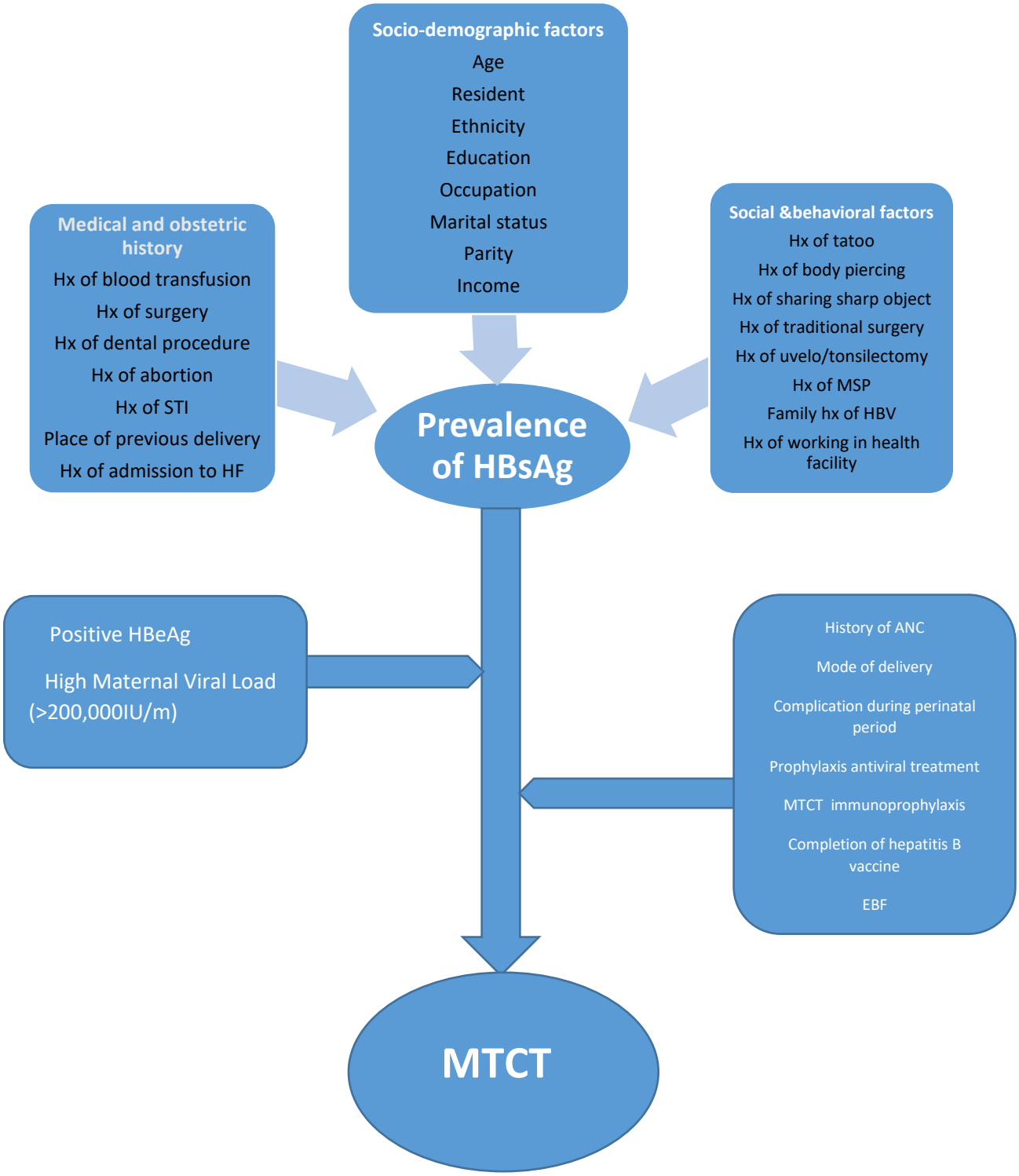
Across countries, comparison between children born before and after the introduction of hepatitis B vaccination programs consistently show a reduced prevalence of hepatitis B surface antigen (HBsAg) in the post-vaccine era. This decline occurred *regardless of the specific vaccination*

schedules used. Longitudinal studies also confirm a steady decrease in HBsAg prevalence over time, particularly in younger age groups. These findings strongly suggest that hepatitis B vaccination efforts have had a substantial impact on reducing HBV transmission among children globally (Marjenberg *et al*., 2022).

According to CDC report, 14 countries in Africa (30%) had implemented the timely Hep B-BD vaccine with over half located in West Africa. Regional coverage increased from 10-18% in between 2016-2022. During this period, Algeria& Cabo Verde reached Hep B-BD coverage of ≥90% and Namibia& Senegal achieved ≥50% coverage. Ethiopia planned to incorporate Hep B-BD into national EPI schedule by 2022. But it has not been implemented yet. In addition other countries still have low performance. Developing countries including Ethiopia fall short of the target. Despite the high burden, most hospitals in Ethiopia have no protocol in place to address the problem. A cross sectional study showed only 42.5% of exposed neonates received first dose of hepatitis B in Southwest Ethiopia. According to WHO/UNICEF only 72% of infants completed the three additional doses of the vaccine in 2023 (EPHI, 2021; Demissie *et al*., 2023; CDC, 2024; WHO, 2024).

Public health education, particularly pregnant women, should be given on means of transmission, risk factors and importance of immunization. Strengthening health infrastructure, implementation of national guideline and continuous monitoring are keys for sustained control of hepatitis B infection.

3.7 Conceptual Framework



4. MATERIALS AND METHOD

4.1 Study Area

The study was conducted in two governmental hospitals and one health center (HC), namely Gambella General Hospital (GGH), Gambella Town Primary Hospital (GTPH), and Newland health center (NLHC), found in Gambella Town. Gambella is the capital city of Gambella region of Ethiopia located in the South Western Ethiopia. The region is bordered by South Sudan in the west, Oromia region in the northeast, and SNNPR in the southeast. The area of the region is 29,783 square kilometers with estimated population of more than 400,000. The region has four administrative zone. Within these zone, there are 13 woreda. Out of the 5 indigenous ethnic groups, the Nuer and the Anyuak are the dominant groups mostly located in the city (Central Statistical Agency, 2013).

According to MOH, Gambella has 5 hospitals (two located in town), 29 health centers, and 142 health posts (MOH, 2024).

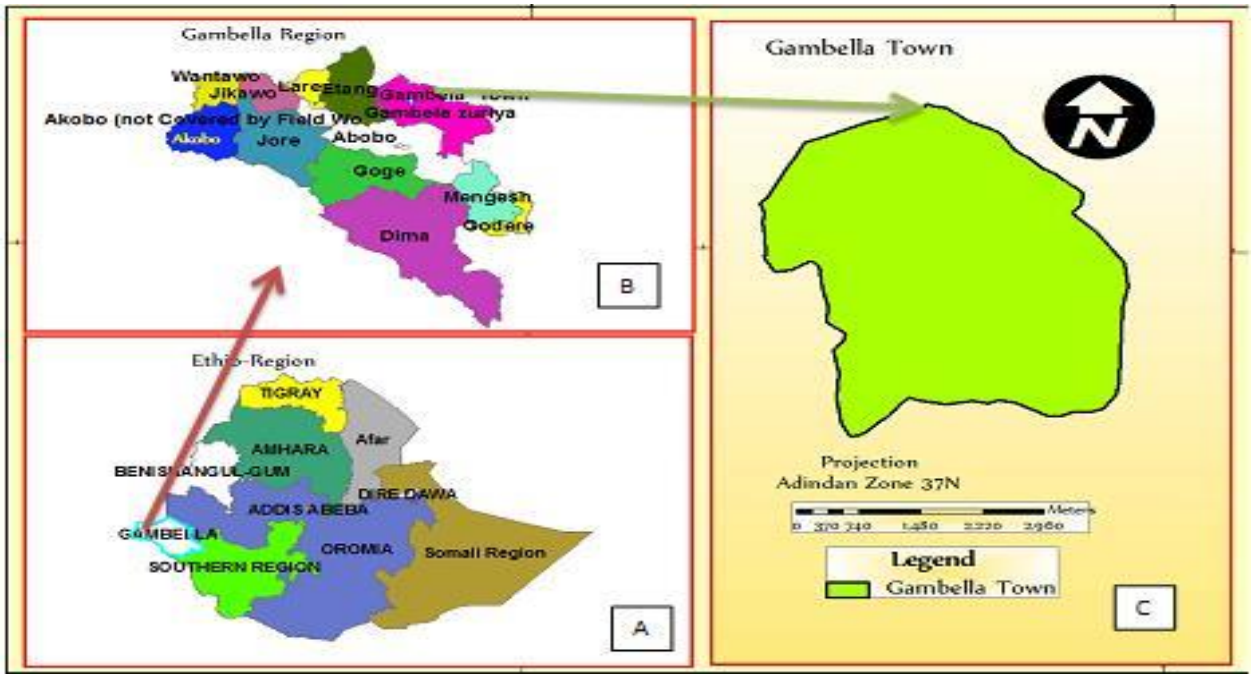


Figure 5. Map of the study area

Source- (Asebe *et al*., 2014)

4.2 Study Design

Health facilities based cross-sectional study was carried out from January to May 2025.

4.3 Study Population

All mothers attending EPI clinic to get their infants vaccinated and all exposed infants within the study period.

4.4 Inclusion and Exclusion Criteria

All mothers who attended EPI to get their infants vaccinated at the hospitals and HC within the study period were included.

All infants between the age of 9 and 15 months born from positive hepatitis B mother were included despite their immunization status.

HIV positive mothers and those who have mental health issues were excluded.

4.5 Sample Size Determination

Sample size was determined by using single population proportion formula based on the following consideration: estimated proportion taken as the rate of mother to child transmission of HBV infection among delivering mothers in Tigray region of 30.9%, 95% Confidence Interval, 5% margin of error (Kiros *et al*., 2020).

$$n = Z_{\alpha/2}^{2} P (1-P)/ d^2$$

The final sample size found appropriate was 328 and after adding 5% nonresponse rate, the final sample size was 350.

4.6 Sampling Procedure

Total of 350 study participants were allocated proportionally based on careful analysis of previous one year data of EPI visit to each health facility. Then it was subjected to some adjustment due to holidays on days of EPI visit and recent house to house campaign. Systemic random sampling was applied to select each participants. Unique code was used for registration of both mothers and infants.

4.7 Data Collection

Informed written consent and assent was taken after providing proper explanation about the purpose and procedure of the study (Appendix E-H). Data on sociodemographic characteristics and associated factors were collected using a structured questionnaire (Appendix A-D). The questionnaire was filled by principle investigator through face to face interview. The interview was conducted in private room to keep the participant's privacy as much as possible.

4.8 Laboratory Procedure

Maternal HBV infection was determined by presence of HBsAg. Samples that tested positive for HBsAg were further tested for HBeAg and HBV DNA to assess the risk of MTCT. Exposed infants were screened for HBsAg and further HBV VL determination to confirm HBV status of the infant.

4.8.1 Blood Sample Collection, Transportation and Storage

5ml of venous blood were collected from each selected mothers by experienced senior laboratory technologists. All plasma samples were separated within 5-min of sample collection by centrifugation of whole blood at 3000rpm for 5min. In Newland health center where centrifuge machine were not available, the serum sample was separated after clot was formed naturally. Rapid HBsAg test for mothers were performed within 10-15min of collection to deliver the result and proceed to collect blood sample from the infant if mother's result was positive. The HBsAg positive plasma samples were transferred to cryotube and stored at -20^{o}c and transported to Addis Ababa (ICL and ALIPB) for HBeAg and HBV DNA determination maintaining the cold chain. Whole blood and DBS samples were collected by finger prick from all exposed infants. Rapid HBsAg test were performed immediately after collection. After proper preparation of DBS, it was stored at room temperature till transportation. When the duration of storage was greater than 2 week, it was stored at -20^{o}c till testing (Appendix I).

4.8.2 Laboratory Examination

All plasma/serum samples from the mothers were tested for HBsAg using RDT (Beright diagnostic kit, Hangzhou Alltest Biotech, Hangzhou, P.R.China) following the manufacturer's instruction. The test has >99.9% sensitivity and 99.4% specificity. HBsAg rapid test for exposed infants was done using Bioline™ HBsAg WB kit (Abbott Diagnostics Korea Inc., Republic of Korea) following

manufacturer's instruction. The test kit has 100% sensitivity and 100% specificity. Positive maternal plasma sample was further tested for HBeAg using Abbott Alinity i (Abbott Diagnostics, Wiesbaden, Germany) following the manufacturer's protocol. It uses chemiluminescent microparticle immunoassay. The test has 100% sensitivity and 100% specificity.

Maternal HBV DNA detection was done using the Xpert ®HBV viral load test (Cepheid, Sweden) which has a lower limit detection (LLD) of 10IU/ml. Fully automated DNA extraction, amplification and detection was performed following the manufacturer's instruction. Infant HBV DNA was measured using the same kit. According to the manufacturer instruction each circle of Whatman 903 card holds 75-80µl of whole blood, therefore it requires correction factor of 23.3 to get the accurate viral load. The results were expressed as HBV DNA copies/ml (IU/ml) or HBV not detected. Gene Xpert HBV viral load test quantify HBV DNA in both plasma and DBS samples accurately with high sensitivity as compared to other gold standard assays, particularly in case for sub-Saharan Africa. The limit of detection of DBS in combination of Xpert HBV viral load assay is around 2 log10 IU/ml (Ceesay *et al*., 2024).

Principles, preparations and procedures of all laboratory tests used in this study. Precautions and safe waste/biohazard handling were applied according to manufacturer's instruction throughout all procedure (Appendix J-N).

4.9 Quality Assurance

The questionnaire that was prepared in English was translated into Nuer, Anuak, and Amharic language which was again translated back to English (Appendix A-D). Modification on questionnaire were made after conducting the pretest. Local translator was used when there was language barrier. Close supervision was done and standard operating procedure (SOP) were strictly followed during blood sample collection, storage, transportation and analytical process. Positive and negative control samples were run to assess the performance of test kit as internal quality control. Known positive and negative plasma samples for HBsAg was obtained from the respective hospitals. The completeness and consistency of questionnaire was checked by principal investigator on each day of data collection.

4.10 Operational Definition

Mother-to-child transmission of HBV is defined as a positive HBsAg test and or detectable viral load in a child born to a mother with HBsAg (Yao *et al.*, 2022).

Exposed infant is an infant born from HBsAg positive mother (CDC, 2024).

4.11 Data Analysis

After checking the completeness and consistency, data were coded and entered into excel and then exported to STATA version 20 for analysis. Descriptive statistical analysis was used to summarize the data using frequency tables and proportions. Bivariate logistic regression analysis was carried out to see the association between HBsAg serostatus and independent variables. Variables having a P-value less than 0.25 were included in the multivariate logistic regression analysis at a 95% confidence interval. Then, multivariate logistic regression was done to control for possible confounders and identify the true effect of the selected predictor variables. Finally, the strength of association between the outcome and predictor variables was assessed using an adjusted odd ratio (AOR) with 95% confidence interval, and the significance of the association was declared at a P-value of less than 0.05. The model adequacy was checked using the Hosmer-Lemeshow test to show the goodness of fitness. Multicollinearity between the independent variables was checked using the variance inflation factor.

5. ETHICAL CONSIDERATIONS

Ethical clearance was obtained from ethical review committee of Aklilu Lemma Institute of Pathobiology institutional review committee, Addis Ababa University. The ethical letter was submitted to Gambella Regional Health Bureau. Then official support letter was written to each health facilities. Then official permission was sought from Gambella General Hospital, Gambella Town Primary Hospital, and Newland health center's administration. Written informed consent was obtained after informing the purpose and importance of the study to each participant. To ensure the confidentiality of the participant's information, a code was used instead of the name of the participants. Participants were interviewed alone to maintain privacy. Individual test results were communicated with the attending physician for further care as per the national management guideline and health education were given to clients on management, need for regular follow up and preventive measures to be taken.

6. Result

A total of 350 mothers were included in this study, with overall response rate of 99%. The mean age of participants was 26yrs (SD±4.3) ranging between 17 and 39yrs. Majority (54%) of participants were between the age of 25-34yrs. Among the major ethnicity, 128(36.6%) and 227(7.7%) of participants were Nuer and Anuak respectively. Regarding their residence, 347(99.1%) of respondents resided in urban area. 242(69.1%) of the respondents had educational level of secondary and above and 263(75.1%) respondents were housewives. Regarding their marital status, 342(97.7%) were married and 214(61.1%) were multiparous. The mean age of exposed infants was 11.4 month (SD±2.8) and ranged between 9 and 15 months. 347(99.4%) of infants completed 3 doses of hepatitis B vaccine according to EPI schedule. 1(0.3%) and 2(0.6%) of them were not vaccinated and did not complete their vaccine. From the 27(1 twin infants) exposed infants, 18(66.7%) of them had no MTCT immunoprophylaxis, whereas 6(22.2%) received both HepB-BD &HBIG respectively. 2(7.4%) and 1(3.7%) of them received only HepB-BD and HBIG respectively (Table 2).

Table 2. Socio-demographic characteristics of perinatal mothers attending EPI clinic at Gambella town, South-western Ethiopia, 2024-2025 (n=350)

No.	Variable	Category	Frequency	Percentage
1.	Age	15-24	137	39.1
		25-34	189	54
		35-44	24	6.9
2.	Ethnicity	Nuer	128	36.6
		Anuak	27	7.7
		Others	195	55.7
3.	Residence	Urban	347	99.1
		Rural	3	0.9
4.	Religion	Orthodox	108	30.9
		Muslim	21	6.0
		Protestant	154	44.0
		Others	67	19.1
5.	Educational level	No formal education	20	5.7
		Primary	88	25.1
		Secondary and above	242	69.1
6.	Occupation	Employed	43	12.3
		Private sector	24	6.9

		House wife	263	75.1
		Student	20	5.7
7.	Marital status	Married	342	97.7
		Unmarried	8	2.3
8.	Parity	Nulliparous	136	38.9
		Multiparous	214	61.1
9.	Household income	No- 4999	83	23.7
		5000-9999	125	35.7
		≥10000	142	40.6

Maternal HBsAg Prevalence

The overall HBsAg seroprevalence among mothers was 7.4% (26/350) (95% CI: 5.1%-10.7%). Among those HBV positive mothers, 14(53.9%) were from Gambella Town primary hospital, 9(34.6%) were from Gambella General Hospital and 3(11.5%) were from Newland health center. The proportion of HBV among age group of 25-34 was 61.5%. 50% of HBV positive mothers had Nuer ethnicity and 92.3% resided in urban area. Surprisingly, the proportion of HBV increased as their eduational level increased. Majority (65.4%) of infected mothers were housewives. All HBV positive mothers were married and 76.9% were multiparous (Table 3).

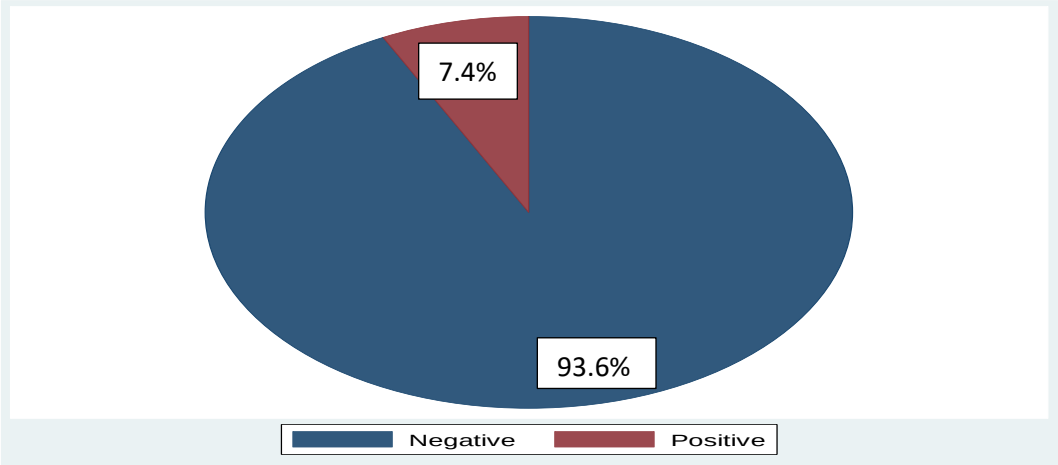


Figure 6. Prevalence of HBsAg among mothers attending EPI at Gambella health facilities, Gambella, 2025 (n=350)

Table 3. Distribution of HBsAg among mothers attending EPI by socio-demographic characteristics at Gambella health facilities, Gambella, 2025, (n=350)

No.	Variable	Category	Status of HBsAg of mothers	
			Positive (%)	Negative (%)
1.	Age	15-24	8 (5.8)	129 (94.2)
		25-34	16 (8.5)	173 (91.5)
		35-44	2 (8.3)	22 (91.7)
2.	Ethnicity	Nuer	13 (10.2)	115 (89.8)
		Anuak	4 (14.8)	23 (85.2)
		Others	9 (4.6)	186 (95.4)
3.	Residence	Urban	24 (6.9)	323 (93.1)
		Rural	2 (66.7)	1 (33.3)
4.	Religion	Orthodox	5 (4.6)	103 (95.4)
		Muslim	2 (9.5)	19 (90.5)
		Protestant	14 (9.1)	140 (90.9)
		Others	5 (7.5)	62 (92.5)
5.	Educational level	No formal education	1 (5)	19 (95)
		Primary	9 (10.2)	79 (89.8)
		Secondary& above	16 (6.6)	226 (93.4)
6.	Occupation	Employed	5 (11.6)	38 (88.4)
		Private sector	2 (8.3)	22 (91.7)
		House wife	17 (6.5)	246 (93.5)
		Student	2 (10)	18 (90)
7.	Marital status	Married	26 (7.6)	316 (92.4)
		Unmarried	0 (0)	8 (100)
8.	Parity	Nulliparous	6 (4.4)	130 (95.6)
		Multiparous	20 (9.35)	194 (90.65)
9.	Household income	No- 4999	6 (7.2)	77 (92.8)
		5000-9999	9 (7.2)	116 (92.8)
		≥10000	11 (7.75)	131 (92.25)

Table 4. Frequency and distribution of risky clinical, cultural, behavioral and institution related factors to HBV infection among mothers attending EPI at Gambella health facilities, Gambella Town, 2025, (n=350)

No.	Characteristics	Category	Frequency (%)	Status of HBsAg of mothers	
				Positive (%)	Negative (%)
1.	History of blood transfusion	Yes	8 (2.3)	2 (25)	6 (75)
		No	342 (97.7)	24 (7.0)	318 (93)
2.	History of surgery	Yes	39 (11.1)	5 (12.8)	34 (87.2)
		No	311 (88.9)	21 (6.75)	290 (93.25)
3.	History of dental procedure	Yes	56 (16)	3 (5.4)	53 (94.6)
		No	294 (84)	23 (7.8)	271 (92.2)
4.	History of abortion	Yes	42 (12)	9 (21.4)	33 (78.6)
		No	308 (88)	17 (5.5)	291 (94.5)
5.	History of STI	Yes	113 (32.3)	15 (13.3)	98 (86.7)
		No	237 (67.7)	11 (4.6)	226 (95.4)
6.	Previous history of liver disease	Yes	12 (3.4)	4 (33.3)	8 (66.7)
		No	338 (96.6)	22 (6.5)	316 (93.5)
7.	History of admission	Yes	105 (30)	12 (11.4)	93 (88.6)
		No	245 (70)	14 (5.7)	231 (94.3)
8.	History of tattoo	Yes	64 (18.3)	5 (7.8)	59 (92.2)
		No	286 (81.7)	21 (7.3)	265 (92.7)
9.	History of body piercing	Yes	326 (93.1)	26 (8)	300 (92)
		No	24 (6.9)	0 (0)	24 (100)
10.	History of sharing sharp object	Yes	244 (69.7)	20 (8.2)	224 (91.8)
		No	106 (30.3)	6 (5.7)	100 (94.3)

11.	History of traditional surgery	Yes	129 (36.9)	4 (3.1)	125 (96.9)
		No	221 (63.1)	22 (9.95)	199 (90.05)
12.	History of uvelectomy/ Tonsillectomy	Yes	53 (15.1)	2 (3.8)	51 (96.2)
		No	297 (84.9)	24 (8.1)	273 (91.9)
13.	History of MSP	Yes	108 (30.9)	18 (16.7)	90 (83.3)
		No	242 (69.1)	8 (3.3)	234 (96.7)
14.	Family history of HBV	Yes	56 (16)	9 (16.1)	47 (83.9)
		No	294 (84)	17 (5.8)	277 (94.2)
15.	History of working in health facility	Yes	5 (1.4)	1 (20)	4 (80)
		No	345 (98.6)	25 (7.25)	320 (92.75)
16.	ANC	Yes	346 (98.9)	26 (7.5)	320 (92.5)
		No	4 (1.1)	0(0)	4 (100)
17.	MOD	Vaginal	319 (91.1)	23 (7.2)	296 (92.8)
		Instrumental	2 (0.6)	0 (0)	2 (100)
		Cesarean section	29 (8.3)	3 (10.3)	26 (89.7)
18.	Complication during labor and delivery	Yes	44 (12.6)	3 (6.8)	41 (93.2)
		No	306 (87.4)	23 (7.5)	283 (92.5)
19.	Place of previous delivery	Home	4 (1.1)	0 (0)	4 (100)
		HF	323 (92.3)	22 (6.8)	301 (93.2)
		Both	23 (6.6)	4 (17.4)	19 (82.6)
20.	HBV-screened during last pregnancy	Yes	55 (15.7)	10 (18.2)	45 (81.8)
		No	34 (9.7)	0 (0)	34 (100)
		Don’t know	261 (74.6)	16 (6.1)	245 (93.9)

Maternal HBeAg prevalence and maternal VL

Among the HBsAg positive mothers, only 1(3.85%) were HBeAg positive. The median maternal HBV DNA was 219 IU/ml (interquartile range: 62-745) and ranged between <10 and 15,000 IU/ml.

Infant HBsAg and VL

All exposed infants (27) born from HBsAg positive mothers were HBsAg negative. And had no detectable viral load.

Table 5. Summary of maternal and exposed infant laboratory result and vaccine status

Exposed infant		Frequency (%)	Maternal		Frequency (%)
HBsAg	Positive	0	HBeAg	Positive	1 (3.85)
	Negative	27(100)		Negative	25 (96.15)
VL	Detectable	0	VL	<200,000	24 (92.3)
	Not detectable	25 (92.6)		>200,000	0
Immunoprophylaxis	Hep B-BD	2 (7.4)			
	HBIG	1 (3.7)			
	Both	6 (22.2)			
	None	18 (66.7)			

Risk factors associated with HBV infection

Bivariate and multivariate logistic regression analysis were conducted to evaluate the association between hepatitis B infection in mothers and different socio-demographic and other potential predictor variables. In bivariate analysis, residency, ethnicity, parity, history of blood transfusion, history of surgical procedure, history of abortion, history of STI, history of admission to health center, history of traditional surgical procedure, history of tonsillectomy/uvelectomy, history of

multiple sexual partner, and family history of HBV were factors significantly associated with HBV infection.

After adjusting for the effect of confounding variables using multivariate logistic regression at $p<0.05$. Three factors were significantly associated with HBV infection in mothers; history of abortion, history of multiple sexual partner and family history of HBV.

Mothers who had history of abortion were 3.5 times more likely to be infected than those with no abortion (AOR=3.5; 95% CI: 1.2-10.4; P=0.026). Those with history of MSP were 5.5 times more likely to be infected (AOR=5.5; 95% CI: 1.7-17.8; P=0.004). Mothers who had family member infected with HBV were 3.7 times more likely to be infected than their counter parts (AOR=3.7; 95% CI: 1.3-10.9; P=0.02)

Table 6. Statistical association of predictor variables with HBsAg seropositivity among mothers attending EPI at Gambella health facilities, Gambella Town, southwestern Ethiopia, 2024-2025 (n=350)

Variable	**Category**	**HBsAg**		**COR (95%CI)**	**AOR (95% CI)**	**P-value**
		Pos	Neg			
Age	15-24	8	129	0.7 (0.1-3.4)	* *	*
	25-35	16	173	0.9 (0.2-4.7)		
	36-45	2	22	1		
Residence	Urban	24	323	0.04 (0.003-0.4)	0.04 (0.002-1.2)	0.06
	Rural	2	1	1	1	
Ethnicity	Nuer	13	115	2.3 (0.97-5.6)	0.7 (0.2-2.6)	0.6
	Anuak	4	23	3.6 (1.02-12.6)	0.6 (0.1-3.4)	
	Others	9	186	1	1	
Education	No formal	1	19	1	* *	*
	Primary	9	79	2.2 (0.3-18.1)		
	Secondary& above	16	226	1.3(0.2-10.7)		
Occupation	Employed	5	38	1.2 (0.2-6.7)	* *	*
	Private sector	2	22	0.8 (0.1-6.4)		
	House wife	17	246	0.6 (0.1-2.9)		
	Student	2	18	1		
Parity	Nulliparous	6	130	0.5 (0.2-1.1)	0.4 (0.1-1.1)	0.08
	Multiparous	20	194	1	1	
Income	No-4999	6	77	1	* *	*
	5000-9999	9	116	0.99 (0.3-2.9)		
	>10000	11	131	1.1 (0.4-3.0)		
Blood transfusion	Yes	2	6	1	1	0.07
	No	24	318	0.2 (0.04-1.2)	0.1 (0.01-1.2)	
Surgery procedure	Yes	5	35	2.0 (0.7-5.7)	1.7 (0.5-6.6)	0.4
	No	21	289	1	1	
Dental procedure	Yes	3	54	1	* *	*
	No	23	270	1.5 (0.4-5.3)		
History of abortion	Yes	9	33	4.7 (1.9-11.3)	3.5 (1.2-10.4)	0.026

	No	17	291	1	1		
History of STI	Yes	15	92	1	1		0.25
	No	11	232	0.3 (0.1-0.7)	0.6 (0.2-1.5)		
History of admission	Yes	12	91	1	1		0.3
	No	14	233	0.5 (0.2-1.0)	0.6 (0.2-1.6)		
History of tattoo	Yes	5	59	1	*	*	*
	No	21	265	0.9 (0.3-2.6)			
Sharing sharp object	Yes	20	225	1.5 (0.6-3.8)	*	*	*
	No	6	99	1			
Traditional surgery	Yes	4	125	0.3 (0.1-0.9)	0.3 (0.1-1.1)		0.07
	No	22	199	1	1		
Uvelectomy Tonsillectomy	Yes	2	51	1	1		0.7
	No	24	273	2.2 (0.5-9.8)	0.7 (0.1-4.0)		
History of MSP	Yes	18	90	5.85 (2.5-13.9)	5.5 (1.7-17.8)		0.004
	No	8	234	1	1		
Family history of HBV	Yes	9	46	3.1 (1.3-7.4)	3.7 (1.3-10.9)		0.02
	No	17	278	1	1		
Working in health facility	Yes	1	8	1	*	*	*
	No	25	316	0.6 (0.08-5.3)			

STI- sexually transmitted infection, MSP-multiple sexual partner,COR- crude odds ratio, AOR- adjusted odds ratio, CI- confidence interval, P-value<0.05 considered significant

7. Discussion

Our study assessed HBsAg seroprevalence, mother to child transmission and associated risk factors among mothers attending EPI at health facilities found in Gambella town, Southwestern Ethiopia.

The study findings revealed the overall HBsAg seroprevalence of 7.4%. According to WHO, the level of endemicity between 2 and 8% is intermediate endemicity. Therefore, the region is classified as high intermediate endemic region (WHO, 2024). The status of HBV infection among the mothers were confirmed to be Chronic Hepatitis B by tracing back their ANC medical report. All mothers had record of positive HBsAg during their ANC visit, fulfilling the criteria for chronic hepatitis B (HBsAg persisted >6 month).

The prevalence of HBV in our study is comparable with studies done in different parts of Ethiopia: the finding is comparable with the prevalence in Hawassa (6.6%) (Kassaw *et al*., 2022), Hararghe(8%) (Umer *et al*., 2023), Wolaita(7.5%) (Tadesse *et al*., 2022), Gambella(7.9%) (Tanga *et al*., 2019), as well as in Chad(7.2%) (Debsikreo *et al*., 2023), Burkina Faso(6.5%) (Ouoba *et al*., 2023), Nigeria(7.9%) (Ukpe *et al*., 2023).

It has higher prevalence than reported in Silte zone, southern Ethiopia (3.3%) (Argaw *et al*., 2022), Wollega(2.4%) (Dabsu and Ejeta, 2018), Addis Ababa(3%) (Arefaine *et al*., 2023), Ambo(4.9%) (Wakjira *et al.*, 2022). Similarly when compared to other countries, our study result is higher than results reported from Somalia (4.1%) (Dahie and Heyle, 2017), Bulgaria(2.26%) (Tsankova *et al*., 2016), Pakistan(2.78%) (Jamil *et al*., 2018), India(2.04) (Ruchika Garg *et al.*, 2017). This variation might be due to the difference in sample size, cultural & behavioral differences, level of awareness, access to antenatal screening services.

In contrast, it is lower than studies reported in Debre Markos, Northen Ethiopia(8.3%) (Demeke *et al*., 2021), 10.9% in Attat hospital, Southern Ethiopia (Geda *et al*., 2021), eastern Tigray(10.4%) (Abay *et al*., 2024). Tigray(11.6% (Kiros *et al*., 2020). Reports from Gambia (9.2%) (Bittaye et al., 2019), Cameroon(9.7%) (Frambo *et al*., 2014), South Sudan(11%) (Kirbak et al., 2017) are higher than our finding. The difference in seroprevalence might be due to geographic setting, sample size variation, study area.

The prevalence of HBsAg was high among the age group 25-34 (61.5%) followed by 15-24 age group. Though age was not significantly associated with HBV, the result suggests that women in these age group are sexually active which prone them to acquiring the infection. This finding is consistent with a report in Eastern Ethiopia (Umare *et al*., 2016). But in contrast with a report from Jimma (Awole and Gebre-Selassie, 2005).

Based on our analysis, history of abortion, history of MSP and family history of HBV were significant predictors of HBV infection. Mothers who had history of abortion has almost 4 times increased chance of acquiring HBV infection than mothers who had no abortion. Similar association were reported from Addis Ababa (Genetu *et al*., 2022), Gambella (Tanga *et al*., 2019) , Hararghe (Mamuye *et al.*, 2020). This association strongly indicate the likelihood undergoing mostly invasive procedures in non-sterile environment which in turn increase the risk of exposure to infected blood and instruments. Mothers who had multiple sexual partner had approximately 6 times increased risk of developing HBV infection. This strong association supports the understanding that having multiple sexual contact is a major route of transmission. The risk increase with the number of exposure. This finding is consistent with reports from Bahir Dar (Gedefaw *et al.*, 2019), Yirgalem (Amsalu *et al*., 2018), Gambella (Tanga *et al*., 2019). Those mothers who had family history of HBV were 4 timely more likely to develop HBV infection than those who had no family history. This finding could explain the risk of horizontal transmission of HBV infection through sharing sharp objects or close physical contact with infected blood or body fluids. Similar association reported from Debre Markos (Demeke *et al.*, 2021), Jigjiga (Roble *et al*., 2021), Amahara(Dagnew *et al*., 2020).

The study reported that only 1(3.85%) of CHB mothers were positive for HBeAg. This finding is lower than reports from Ethiopia (15.2%) (Arefaine *et al*., 2024), Silte zone (18.2%) (Argaw *et al*., 2022), Yirgalem (38.8%) (Amsalu *et al*., 2018), Addis Ababa (12.5%) (Tegegne *et al*., 2014), Libya (21.7%) (El-Magrahe et al., 2010), Nigeria (14.59%) (Olakunde *et al*., 2021), Turkiye (6.2%) (Kuru *et al*., 1996), Nepal (40%) (Shedain *et al*., 2017). But in contrast to our finding, Jordan has lower HBeAg prevalence (0.1%) (Batayneh and Bdour, 2002).

According to Hadziyannis' review (2011), the dominance of HBeAg negative CHB among African population is attributed to major loss of HBeAg (annual rate of 14-16%) leading majority to HBeAg negative CHB by their second decade of life. This leaves <5% of HBsAg positive young

adults and women of reproductive age group be HBeAg positive. It has also direct associated with the predominant HBV genotype. The review corresponds with our findings. Phase of chronic hepatitis B is another important factor for vertical transmission (Belopolskaya *et al*., 2021). From the results we found, the study participants are more likely to be in inactive HBsAg carriage or HBeAg negative phase of CHB, making the risk of vertical transmission very low which in our case, no MTCT.

Viral load of our study participants were far below the requirement for MTCT. In general, the risk of MTCT of our study is categorized under low risk of vertical transmission. In correspondence to our finding Boucheron's review stated the rate of MTCT as extremely low, only 0.04% (95% CI: 0.00-0.25) despite immunoprophylaxis, when maternal HBV DNA level was below 5.3 log10 IU/ml (equivalent to 200,000 IU/ml) (Boucheron *et al*., 2021).

Our study report revealed no (0%) mother to child transmission with all exposed infants being negative for HBsAg and had no detectable viral load, despite their differences in immunization status. The finding aligns was to Senegal (0%) (Marinier *et al*., 1985), Nigeria (0%) (Ndububa *et al*., 2022). Our finding is lower than previous reports from Ethiopia (7.1%) (Arefaine *et al*., 2024), Tigray (30.9%) (Kiros *et al*., 2020), Ethiopia (10.1%) (Johannessen *et al*., 2021), Libya (60.9%) (El-Magrahe *et al*., 2010), Egypt (51.8%) (Badawy and El-Salahy, 2000), India (29.4%) (Chakravarti et al., 2005), China (5.21%) (Chen *et al*., 2018). The variation in reports of vertical transmission rates from HBsAg positive mothers to their infants depends on several factors, including viral load, HBeAg, HBV variants, difference in the age at assessment of HBV infection of infants, sensitivity and accuracy of diagnostic tests and sample size.

In most studies done on MTCT in Ethiopia, the age of assessment of HBV infection was at birth which makes it difficult to draw conclusion on HBV infection status of the infants and further bringing them to comparison with current study. According to Breakwell *et al*, there is no association between infant's HBsAg status at 9 month and the presence of HBsAg in cord blood during delivery (Breakwell *et al*., 2017). Still our report was far below than studies done at similar age of the infants. Since there was no mother to child transmission in this study, risk factors associated with MTCT was not assessed.

Since it was cross-sectional study among mothers, we were unable to ascertain when the actual HBV infection had taken place. Although the frequency of spontaneous HBsAg loss is low (1% per year), there could be a possibility of missing HBsAg positive mothers and infant.

8. Conclusion and recommendation

Gambella has intermediate seroprevalence of HBV among mothers. Abortion, history of multiple sexual partner and family history of HBV were risk factors associated with HBV. Almost all infected mothers had chronic hepatitis B with negative HBeAg and low viral load predominance, classifying them under low risk. There was no MTCT in this study. HBeAg and VL were factors strongly related to the risk of MTCT.

Therefore, based on the above conclustions the following recommendations were forwarded:

- HBV screening should be strengthened in all antenatal care providing health facilities and organize easy access to treatment of HBV.
- Safe and comprehensive abortion care should be delivered to all mothers in need.
- Mothers sould be encouraged to receive the hepatitis B.
- Provision of health education about HBV and its preventive methods should be given.
- Further molecular characterization should be conducted.

4 REFERENCE

Abay, G.K., Gebrewahd, A., Berhe, B., Belay, G., Tesfanchal, B., Kahsay, T., Fseha, B., Tadesse, S., Birhane, M., Gebre, M., 2024. Sero-prevalence and risk factors of hepatitis B virus infection among pregnant women attending antenatal clinics in Adigrat General Hospital, Eastern Tigrai, Northern Ethiopia.

Abebe, A., Nokes, D.J., Dejene, A., Enquselassie, F., Messele, T., Cutts, F.T., 2003. Seroepidemiology of hepatitis B virus in Addis Ababa, Ethiopia: transmission patterns and vaccine control. Epidemiology & Infection 131, 757–770.

Alemu, A.A., Zeleke, L.B., Aynalem, B.Y., Kassa, G.M., 2020. Hepatitis B virus infection and its determinants among pregnant women in Ethiopia: a systematic review and meta-analysis. Infectious diseases in obstetrics and gynecology 2020.

Amsalu, A., Ferede, G., Eshetie, S., Tadewos, A., Assegu, D., 2018. Prevalence, infectivity, and associated risk factors of hepatitis B virus among pregnant women in Yirgalem Hospital, Ethiopia: implication of screening to control mother-to-child transmission. Journal of pregnancy 2018.

Ansari, A., Vincent, J.P., Moorhouse, L., Shimakawa, Y., Nayagam, S., 2023. Risk of early horizontal transmission of hepatitis B virus in children of uninfected mothers in sub-Saharan Africa: a systematic review and meta-analysis. The Lancet Global Health 11, e715–e728. https://doi.org/10.1016/S2214-109X(23)00131-6

Arefaine, M., Johannessen, A., Teklehaymanot, T., Mihret, A., Alemayehu, D.H., Osman, M., Mulu, A., Berhe, N., 2024. A prospective, multicenter study of hepatitis B birth-dose vaccine with or without hepatitis B immunoglobulin in preventing mother-to-child transmission of hepatitis B virus in Ethiopia. Vaccine 42, 126461.

Arefaine, M., Johannessen, A., Teklehaymanot, T., Mulu, A., Hailu, D., Mihret, A., Osman, M., Teshay, D., Berhe, N., 2023. Effectiveness of birth-dose vaccine in preventing mother-to-child transmission of hepatitis B virus in Ethiopia. Journal of Hepatology 78, S927–S928.

Argaw, B., Kedir, S., Mustefa, A., Yesse, M., Hussen, L., Abdella, B., Muze, M., Jemal, M., 2022. Sero-prevalence, infectivity, and associated risk factors of hepatitis B virus among pregnant women attending antenatal care in sankura primary hospital, silte zone, Southern Ethiopia, 2021. The Open Microbiology Journal 16.

Asebe, G., Ameni, G., Tafess, K., 2014. Ten years tuberculosis trend in Gambella Regional Hospital, South Western Ethiopia. Malaysian Journal of Medical and Biological Research 1, 18–24. https://doi.org/10.18034/mjmbr.v1i1.371

Asgedom, Y.S., Kassie, G.A., Woldegeorgis, B.Z., Meskele Koyira, M., Kebede, T.M., 2024. Seroprevalence of hepatitis B virus infection and factors associated among pregnant women in Ethiopia: A systematic review and meta-analysis. Womens Health (Lond Engl) 20, 17455057241235881. https://doi.org/10.1177/17455057241235881

Assefa, A., Getie, M., Getie, B., Simeneh, T., Enkobahry, A., 2024. Molecular epidemiology of hepatitis B virus (HBV) in Ethiopia: A review article. Infection, Genetics and Evolution 105618.

Awole, M., Gebre-Selassie, S., 2005. Seroprevalence of HBsAg and its risk factors amoung pregnant women in Jimma, Southwest Ethiopia. Ethiopian Journal of Health Development 19, 45–50.

Ayele, A., Abera, D., Hailu, M., Birhanu, M., Desta, K., 2020. Prevalence and associated risk factors for Hepatitis B and C viruses among refugees in Gambella, Ethiopia. BMC Public Health 20, 721. https://doi.org/10.1186/s12889-020-08893-1

Badawy, H.A., El-Salahy, E., 2000. Materno-foetal transmission of hepatitis B infection. J Egypt Public Health Assoc 75, 357–367.

Bafa, T.A., Egata, A.D., 2020. Seroepidemiological patterns and predictors of hepatitis B, C and HIV viruses among pregnant women attending antenatal care clinic of Atat Hospital, Southern Ethiopia. SAGE Open Medicine 8, 2050312119900870. https://doi.org/10.1177/2050312119900870

Bai, G., Wang, Y., Zhang, L., Tang, Y., Fu, F., 2012. The study on the role of hepatitis B virus X protein and apoptosis in HBV intrauterine infection. Archives of gynecology and obstetrics 285, 943–949.

Bancha, B., Kinfe, A.A., Chanko, K.P., Workie, S.B., Tadese, T., 2020. Prevalence of hepatitis B viruses and associated factors among pregnant women attending antenatal clinics in public hospitals of Wolaita Zone, South Ethiopia. PloS one 15, e0232653.

Batayneh, N., Bdour, S., 2002. Risk of Perinatal Transmission of Hepatitis B Virus in Jordan. Infectious Diseases in Obstetrics and Gynecology 10, 752568. https://doi.org/10.1155/S1064744902000121

Belopolskaya, M., Avrutin, V., Kalinina, O., Dmitriev, A., Gusev, D., 2021. Chronic hepatitis B in pregnant women: Current trends and approaches. World J Gastroenterol 27, 3279–3289. https://doi.org/10.3748/wjg.v27.i23.3279

Bertoletti, A., Hong, M., 2014. Age-Dependent Immune Events during HBV Infection from Birth to Adulthood: An Alternative Interpretation. Front. Immunol. 5. https://doi.org/10.3389/fimmu.2014.00441

Bittaye, M., Idoko, P., Ekele, B.A., Obed, S.A., Nyan, O., 2019. Hepatitis B virus sero-prevalence amongst pregnant women in the Gambia. BMC Infect Dis 19, 259. https://doi.org/10.1186/s12879-019-3883-9

Boucheron, P., Lu, Y., Yoshida, K., Zhao, T., Funk, A.L., Lunel-Fabiani, F., Guingané, A., Tuaillon, E., Holten, J. van, Chou, R., Bulterys, M., Shimakawa, Y., 2021. Accuracy of HBeAg to identify pregnant women at risk of transmitting hepatitis B virus to their neonates: a systematic review and meta-analysis. The Lancet Infectious Diseases 21, 85–96. https://doi.org/10.1016/S1473-3099(20)30593-4

Breakwell, L., Tevi-Benissan, C., Childs, L., Mihigo, R., Tohme, R., 2017. The status of hepatitis B control in the African region. Pan Afr Med J 27, 17. https://doi.org/10.11604/pamj.supp.2017.27.3.11981

CDC. Progress Toward Hepatitis B Control and Elimination of Mother-to-Child Transmission of Hepatitis B Virus — World Health Organization African Region, 2016–2021, 2024 https://www.cdc.gov/mmwr/volumes/72/wr/mm7229a2.htm Accessed on November 20, 2024

Ceesay, A., Drammeh, S., Ndow, G., Jallow, A.O.A., Nyang, H., Bittaye, B., Mendy, F.S., Secka, O., D'Alessandro, U., Shimakawa, Y., Vo-Quang, E., Testoni, B., Thursz, M., Lemoine, M., Chemin, I., 2024. Real-Life Diagnostic Accuracy and Clinical Utility of Hepatitis B Virus (HBV) Nucleic Acid Testing Using the GeneXpert Point-of-Care Test System from Fresh Plasma and Dry Blood Spot Samples in The Gambia. Microorganisms 12, 2273. https://doi.org/10.3390/microorganisms12112273

Chakravarti, A., Rawat, D., Jain, M., 2005. A STUDY ON THE PERINATAL TRANSMISSION OF THE HEPATITIS B VIRUS. Indian Journal of Medical Microbiology 23, 128–130. https://doi.org/10.1016/S0255-0857(21)02654-2

Chang, M.-H., 2007. Hepatitis B virus infection. Seminars in Fetal and Neonatal Medicine, Congenital and Opportunistic Infections 12, 160–167. https://doi.org/10.1016/j.siny.2007.01.013

Chen, T., Wang, J., Qiu, H., Yu, Q., Yan, T., Qi, C., Cao, F., Tian, Z., Guo, D., Yao, N., Yang, Y., He, Y., Zhao, Y., Liu, J., 2018. Different interventional criteria for chronic hepatitis B pregnant women with HBeAg(+) or HBeAg(-): Epidemiological data from Shaanxi, China. Medicine 97, e11406. https://doi.org/10.1097/MD.0000000000011406

Chuang, Y.-C., Tsai, K.-N., Ou, J.-H.J., 2022. Pathogenicity and virulence of Hepatitis B virus. Virulence 13, 258–296. https://doi.org/10.1080/21505594.2022.2028483

Dabsu, R., Ejeta, E., 2018. Seroepidemiology of *Hepatitis B* and *C Virus* Infections among Pregnant Women Attending Antenatal Clinic in Selected Health Facilities in East Wollega Zone, West Oromia, Ethiopia. BioMed Research International 2018, 1–9. https://doi.org/10.1155/2018/4792584

Dagnew, M., Million, Y., Gizachew, M., Eshetie, S., Yitayew, G., Asrade, L., Adefris, M., Moges, F., Tiruneh, M., 2020. Hepatitis B and C viruses' infection and associated factors among pregnant women attending antenatal care in hospitals in the Amhara national regional state, Ethiopia. International journal of microbiology 2020.

Dahie, H.A., Heyle, A.A., 2017. Prevalence of hepatitis B and its associated factors among pregnant women in Mogadishu, Somalia. Archives of Business Research 5.

Debsikreo, N., Mankréo, B.L., Ouangkake, M., Jotham, M., Ndiaye, A.J.S., Leye, N., LO, G., Moukenet, A., Moussa, A.M., Toure-Kane, N.C., 2023. Prevalence of hepatitis B virus and associated factors among pregnant women in the health facilities, N'djamena, Chad.

Demeke, G., Ayalneh, G.M., Shiferaw, A.A., Toru, M., Dilnessa, T., 2021. Sero-Prevalence and Associated Factors of Hepatitis B Virus Among Pregnant Women at North West Ethiopia: An Institution-Based Cross-Sectional Study. IJGM Volume 14, 2799–2805. https://doi.org/10.2147/IJGM.S320711

Demissie, M., Weldekidan, H.A., Yosef, Y., Sori, S.A., Tsega, D., Jiru, H.D., Derribow, A.B., Tetema, M.D., Jima, G.B., Zeleke, F.T., Endeshew, F., Abeje, S., 2023. Timing of Hepatitis B Vaccine Birth Dose in Exposed Newborns, Southwest Ethiopia: A Cross-Sectional Study. SAGE Open Nursing 9, 23779608231187258. https://doi.org/10.1177/23779608231187258

Duri, K., Munjoma, P.T., Mataramvura, H., Mazhandu, A.J., Chandiwana, P., Marere, T., Gumbo, F.Z., Mazengera, L.R., 2023. Antenatal hepatitis B virus sero-prevalence, risk factors, pregnancy outcomes and vertical transmission rate within 24 months after birth in a high HIV prevalence setting. BMC Infect Dis 23, 736. https://doi.org/10.1186/s12879-023-08523-2

El-Magrahe, H., Furarah, A.R., El-Figih, K., El-Urshfany, S., Ghenghesh, K.S., 2010. Maternal and neonatal seroprevalence of Hepatitis B surface antigen (HBsAg) in Tripoli, Libya. The Journal of Infection in Developing Countries 4, 168–170. https://doi.org/10.3855/jidc.609

EPHI, 2021 http://www.dataverse.nipn.ephi.gov.et/bitstream/handle/123456789/2941/cMYP (2021-2025) Accessed on October 2, 2024

FDRE Central Statistical Agency (2013) Population projection of Ethiopia for all regions at wereda level from 2014–2017. Addis Ababa: FDRE Central Statistical Agency

Fiseha, T., Alemayehu ,Ermiyas, Mohammed ,Ousman, Gedefie ,Alemu, Adamu ,Aderaw, Tamir ,Zemenu, and Gebreweld, A., 2023. Reference Intervals of Haematological Parameters for Apparently Healthy Adults in Northeast Ethiopia. International Journal of General Medicine 16, 5309–5321. https://doi.org/10.2147/IJGM.S430751

Frambo, A.A.B., Atashili, J., Fon, P.N., Ndumbe, P.M., 2014. Prevalence of HBsAg and knowledge about hepatitis B in pregnancy in the Buea Health District, Cameroon: a cross-sectional study. BMC Res Notes 7, 394. https://doi.org/10.1186/1756-0500-7-394

Geda, Y.F., Desse, H., Gesesse, M.M., Berhe, T.M., 2021. Hepatitis B surface antigen and associated factors among mothers who had antenatal care contact in Attat Hospital, southern Ethiopia. SAGE Open Medicine 9, 20503121211024462. https://doi.org/10.1177/20503121211024462

Gedefaw, G., Waltengus, F., Akililu, A., Gelaye, K., 2019. Risk factors associated with hepatitis B virus infection among pregnant women attending antenatal clinic at Felegehiwot referral hospital, Northwest Ethiopia, 2018: an institution based cross sectional study. BMC Res Notes 12, 509. https://doi.org/10.1186/s13104-019-4561-0

GE Life Sciences GHL. 903 Protein Saver Card 2016 http://www.gelifesciences.com/webapp/wcs/stores/servlet/catalog/en/GELifeSciences-no/products/AlternativeProductStructure_21577/28416563. Accessed on May 20, 2025

Genetu, K., Abere, K., Tachbele, E., 2022. Magnitudes and Correlates of Human Immunodeficiency Virus, Hepatitis B Virus, and Syphilis among Pregnant Mothers Attending Antenatal Care in Addis Ababa, Ethiopia. Infectious Diseases in Obstetrics and Gynecology 2022, 1–9. https://doi.org/10.1155/2022/6156613

Grüner, N., Stambouli, O., Ross, R.S., 2015. Dried blood spots-preparing and processing for use in immunoassays and in molecular techniques. Journal of visualized experiments: JoVE 52619.

Hadziyannis, S.J., 2011. Natural history of chronic hepatitis B in Euro-Mediterranean and African countries. Journal of hepatology 55, 183–191.

Hoffmann, C.J., Thio, C.L., 2007. Clinical implications of HIV and hepatitis B co-infection in Asia and Africa. Lancet Infect Dis 7, 402–409. https://doi.org/10.1016/S1473-3099(07)70135-4

Hu, J., Liu, K., 2017. Complete and Incomplete Hepatitis B Virus Particles: Formation, Function, and Application. Viruses 9, 56. https://doi.org/10.3390/v9030056

Hwang, E.W., Cheung, R., 2011. Global epidemiology of hepatitis B virus (HBV) infection. North American Journal of Medicine and Science 4.

Jamil, A., Hamid, S., Aziz, Q., 2018. Prevalence of Hepatitis B Among Pregnant Women. Journal of Islamabad Medical & Dental College 7, 128–131.

Johannessen, A., Mekasha, B., Desalegn, H., Aberra, H., Stene-Johansen, K., Berhe, N., 2021. Mother-to-child transmission of hepatitis B virus in Ethiopia. Vaccines 9, 430.

Joshi, S.S., Coffin, C.S., 2020. Hepatitis B and Pregnancy: Virologic and Immunologic Characteristics. Hepatol Commun 4, 157–171. https://doi.org/10.1002/hep4.1460

Kabore, H.J., 2023. Progress Toward Hepatitis B Control and Elimination of Mother-to-Child Transmission of Hepatitis B Virus — World Health Organization African Region, 2016–2021. MMWR Morb Mortal Wkly Rep 72. https://doi.org/10.15585/mmwr.mm7229a2

Kang, W., Ding, Z., Shen, L., Zhao, Z., Huang, G., Zhang, J., Xiong, Q., Zhang, Shuang, Zhang, Shuo, Wang, F., 2014. Risk factors associated with immunoprophylaxis failure against mother to child transmission of hepatitis B virus and hepatitis B vaccination status in Yunnan province, China. Vaccine 32, 3362–3366. https://doi.org/10.1016/j.vaccine.2014.04.045

Kassaw, B., Abera, N., Legesse, T., Workineh, A., Ambaw, G., 2022. Sero-prevalence and associated factors of hepatitis B virus among pregnant women in Hawassa city public hospitals, Southern Ethiopia: Cross-sectional study design. SAGE Open Medicine 10, 20503121221140778. https://doi.org/10.1177/20503121221140778

Keane, E., Funk, A.L., Shimakawa, Y., 2016. Systematic review with meta-analysis: the risk of mother-to-child transmission of hepatitis B virus infection in sub-Saharan Africa. Aliment Pharmacol Ther 44, 1005–1017. https://doi.org/10.1111/apt.13795

Kibret, G.D., Ferede, A., Leshargie, C.T., Wagnew, F., Ketema, D.B., Alebel, A., 2019. Trends and spatial distributions of HIV prevalence in Ethiopia. Infect Dis Poverty 8, 90. https://doi.org/10.1186/s40249-019-0594-9

Kirbak, A.L.S., Ng'ang'a, Z., Omolo, J., Idris, H., Usman, A., Mbabazi, W.B., 2017. Sero-prevalence for Hepatitis B virus among pregnant women attending antenatal clinic in Juba Teaching Hospital, Republic of South Sudan. Pan Afr Med J 26, 72. https://doi.org/10.11604/pamj.2017.26.72.11410

Kiros, K.G., Goyteom, M.H., Tesfamichael, Y.A., Mekonen, H.H., Gebru, T.H., Gebrehiwot, T.G., Teka, Y.H., Abrha, W.A., Tadesse, D.B., 2020. Seroprevalence of Hepatitis B Virus Infection, Mother-To-Child Transmission, and Associated Risk Factors Among Delivering Mothers in Tigray Region, Northern Ethiopia: a Cross-Sectional Study. Infect Dis Ther 9, 901–911. https://doi.org/10.1007/s40121-020-00340-3

Komatsu, H., Inui, A., Sogo, T., Hiejima, E., Tateno, A., Klenerman, P., Fujisawa, T., 2010. Cellular immunity in children with successful immunoprophylactic treatment for mother-to-child transmission of hepatitis B virus. BMC Infectious Diseases 10, 103. https://doi.org/10.1186/1471-2334-10-103

Kourtis, A.P., Bulterys, M., Hu, D.J., Jamieson, D.J., 2012. HIV-HBV coinfection--a global challenge. N Engl J Med 366, 1749–1752. https://doi.org/10.1056/NEJMp1201796

Kuru, U., Turan, O., Kuru, N., Saglam, Z., Ceylan, Y., Nurluoglu, M., Agacfidan, A., 1996. Prevalence of hepatitis B virus infection in pregnant Turkish women and their families. Eur. J. Clin. Microbiol. Infect. Dis. 15, 248–251. https://doi.org/10.1007/BF01591364

Larebo, Y.M., Anshebo, A.A., Abdo, R.A., Behera, S.K., Gopalan, N., 2024. Prevalence of hepatitis B virus infection among pregnant women in Africa: A systematic review and meta-analysis. Plos one 19, e0305838.

Lee, C., Gong, Y., Brok, J., Boxall, E.H., Gluud, C., 2006. Effect of hepatitis B immunisation in newborn infants of mothers positive for hepatitis B surface antigen: systematic review and meta-analysis. BMJ 332, 328–336. https://doi.org/10.1136/bmj.38719.435833.7C

Liang, T.J., 2009. Hepatitis B: The virus and disease. Hepatology 49, S13–S21. https://doi.org/10.1002/hep.22881

Mackie, C.O., Buxton, J.A., Tadwalkar, S., Patrick, D.M., 2009. Hepatitis B immunization strategies: timing is everything. CMAJ 180, 196–202. https://doi.org/10.1503/cmaj.081112

Mamuye, B., Gobena, T., Oljira, L., 2020. Hepatitis B virus infection and associated factors among pregnant women attending antenatal clinics in West Hararghe public hospitals, Oromia region, Ethiopia. Pan African Medical Journal 35.

Marinier, E., Barrois, V., Larouze, B., London, W.T., Cofer, A., Diakhate, L., Blumberg, B.S., 1985. Lack of perinatal transmission of hepatitis B virus infection in senegal, West Africa. The Journal of Pediatrics 106, 843–849. https://doi.org/10.1016/S0022-3476(85)80371-1

Marjenberg, Z., Wright, C., Pooley, N., Cheung, K.W., Shimakawa, Y., Vargas-Zambrano, J.C., Vidor, E., 2022. Hepatitis B surface antigen prevalence and the rates of mother-to-child transmission of hepatitis B virus after the introduction of infant vaccination programs in South East Asia and Western Pacific regions: a systematic review. International Journal of Infectious Diseases 124, 65–75.

Mast, E.E., Margolis, H.S., Fiore, A.E., Brink, E.W., Goldstein, S.T., Wang, S.A., Moyer, L.A., Bell, B.P., Alter, M.J., 2005. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States. MMWR 54, 1–32.

MOH. Gambella Regional Health Bureau, 2024 https://www.moh.gov.et/en/Gambella_Regional_Health_Bureau?language_content_entity=en Accessed on November 23, 2024

Navabakhsh, B., Mehrabi, N., Estakhri, A., Mohamadnejad, M., Poustchi, H., 2011. Hepatitis B virus infection during pregnancy: transmission and prevention. Middle East journal of digestive diseases 3, 92.

Nayagam, S., Shimakawa, Y., Lemoine, M., 2020. Mother-to-child transmission of hepatitis B: What more needs to be done to eliminate it around the world? Journal of Viral Hepatitis 27, 342–349. https://doi.org/10.1111/jvh.13231

Ndububa, D., Kuti, O., Awowole, I., Adekanle, O., Ijarotimi, O., Makinde, O., Adeyemi, A., Anyabolu, C., Ijadunola, M., 2022. Prospective cohort study of prevention of mother to child transmission of hepatitis B infection and 9 months follow-up of hepatitis B-exposed infants at Ile-Ife, Nigeria. BMJ Open 12, e063482. https://doi.org/10.1136/bmjopen-2022-063482

Olakunde, B.O., Adeyinka, D.A., Olakunde, O.A., Uthman, O.A., Bada, F.O., Nartey, Y.A., Obiri-Yeboah, D., Paintsil, E., Ezeanolue, E.E., 2021. A systematic review and meta-analysis of the prevalence of hepatitis B virus infection among pregnant women in Nigeria. PLOS ONE 16, e0259218. https://doi.org/10.1371/journal.pone.0259218

Ott, J.J., Stevens, G.A., Wiersma, S.T., 2012. The risk of perinatal hepatitis B virus transmission: hepatitis B e antigen (HBeAg) prevalence estimates for all world regions. BMC Infect Dis 12, 131. https://doi.org/10.1186/1471-2334-12-131

Ouoba, S., Ko, K., Lingani, M., Nagashima, S., Guingané, A.N., Bunthen, E., Hussain, M.R.A., Sugiyama, A., Akita, T., Ohisa, M., 2023. Intermediate hepatitis B virus infection prevalence among 1622 pregnant women in rural Burkina Faso and implications for mother-to-child transmission. Scientific Reports 13, 6115.

Pan, C.Q., Duan, Z., Bhamidimarri, K.R., Zou, H., Liang, X., Li, J., Tong, M.J., 2012. An Algorithm for Risk Assessment and Intervention of Mother to Child Transmission of Hepatitis B Virus. Clinical Gastroenterology and Hepatology 10, 452–459. https://doi.org/10.1016/j.cgh.2011.10.041

Peng, S., Wan, Z., Liu, T., Li, X., Du, Y., 2018. Cesarean section reduces the risk of early mother-to-child transmission of hepatitis B virus. Digestive and Liver Disease 50, 1076–1080. https://doi.org/10.1016/j.dld.2018.05.004

Roble, A.K., Roba, K.T., Mengistie, B., Abdurke Kure, M., 2021. Seroprevalence of Hepatitis B Virus and Associated Factors Among Pregnant Women Attending Antenatal Care in Public

Health Facilities in Jigjiga Town, Eastern Ethiopia. IJWH Volume 12, 1299–1310. https://doi.org/10.2147/IJWH.S276526

Ruchika Garg, R.G., Asha Nigam, A.N., Saroj Singh, S.S., Richa Singh, R.S., Shikha Singh, S.S., Rekha Rani, R.R., 2017. Seroprevalence of hepatitis B surface antigen among pregnant women in a tertiary care health center of North India.

Sangaré, L., Sombié, R., Combasséré, A.W., Kouanda, A., Kania, D., Zerbo, O., Lankoandé, J., 2009. [Antenatal transmission of hepatitis B virus in an area of HIV moderate prevalence, Burkina Faso]. Bull Soc Pathol Exot 102, 226–229.

Schillie, S., 2017. Prevention of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices. MMWR. Recommendations and reports 67.

Shedain, P.R., Devkota, M.D., Banjara, M.R., Ling, H., Dhital, S., 2017. Prevalence and risk factors of hepatitis B infection among mothers and children with hepatitis B infected mother in upper Dolpa, Nepal. BMC Infect Dis 17, 667. https://doi.org/10.1186/s12879-017-2763-4

Shimakawa, Y., Lemoine, M., Njai, H.F., Bottomley, C., Ndow, G., Goldin, R.D., Jatta, A., Jeng-Barry, A., Wegmuller, R., Moore, S.E., 2016. Natural history of chronic HBV infection in West Africa: a longitudinal population-based study from The Gambia. Gut 65, 2007–2016.

Shimakawa, Y., Veillon, P., Birguel, J., Pivert, A., Sauvage, V., Guillou-Guillemette, H.L., Roger, S., Njouom, R., Ducancelle, A., Amta, P., Huraux, J.M., Adoukara, J.-P., Lunel-Fabiani, F., 2022. Residual risk of mother-to-child transmission of hepatitis B virus infection despite timely birth-dose vaccination in Cameroon (ANRS 12303): a single-centre, longitudinal observational study. The Lancet Global Health 10, e521–e529. https://doi.org/10.1016/S2214-109X(22)00026-2

Singh, J., Sinha, S., 2015. Emerging of RNA Viruses: A Threat of Epidemics Around-The-Clock. International Journal of Applied Biology and Pharmaceutical Technology 6, 80–92.

Sinha, S., Kumar, M., 2010. Pregnancy and chronic hepatitis B virus infection. Hepatology Research 40, 31–48. https://doi.org/10.1111/j.1872-034X.2009.00597.x

Tadesse, M., Tafesse, G., Hajare, S.T., Chauhan, N.M., 2022. Assessment of prevalence of Hepatitis B virus and its associated factors among pregnant women from Wolaita Sodo, Ethiopia. Journal of Clinical Virology Plus 2, 100069.

Tang, J., Zhao, H., 2024. Concerns on the mother-to-child transmission of hepatitis B virus reported in Ethiopia. The Journal of Infection in Developing Countries 18, 1780–1781.

Tanga, A.T., Teshome, M.A., Hiko, D., Fikru, C., Jilo, G.K., 2019. Sero-prevalence of hepatitis B virus and associated factors among pregnant women in Gambella hospital, South Western Ethiopia: facility based cross-sectional study. BMC Infect Dis 19, 602. https://doi.org/10.1186/s12879-019-4220-z

Taye, B.W., Ayenew, G.M., Taye, Z.W., Balew, M., Taye, E.B., 2023. The risk of mother-to-child transmission of hepatitis B virus infection in Ethiopia: A systematic review and meta-analysis. The Journal of Infection in Developing Countries 17, 744–751.

Tegegne, D., Desta, K., Tegbaru, B., Tilahun, T., 2014. Seroprevalence and transmission of Hepatitis B virus among delivering women and their new born in selected health facilities, Addis Ababa, Ethiopia: a cross sectional study. BMC Res Notes 7, 239. https://doi.org/10.1186/1756-0500-7-239

Terrault, N.A., Lok, A.S.F., McMahon, B.J., Chang, K.-M., Hwang, J.P., Jonas, M.M., Brown Jr., R.S., Bzowej, N.H., Wong, J.B., 2018. Update on prevention, diagnosis, and treatment of

chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology 67, 1560–1599. https://doi.org/10.1002/hep.29800

Tesfu, M.A., Habtemariam, T.T., Belay, N.B., 2023. Risk factors associated with Hepatitis B virus infection among pregnant women attending public hospitals in Addis Ababa, Ethiopia. Plos one 18, e0284646.

Tsankova, G.S., Kostadinova, T., Todorova, T.T., 2016. Seroprevalence of hepatitis B among pregnant women in Varna Region, Bulgaria. Journal of Medical Virology 88, 2012–2015. https://doi.org/10.1002/jmv.24543

Ukpe, M.T., Abasiattai, A.M., Utuk, N.M., Umoh, A.V., Ibanga, G.J., 2023. Prevalence of hepatitis B virus infection among antenatal attendees in a university teaching hospital in Southern Nigeria. Adesh Univ J Med Sci Res 5, 9–16. https://doi.org/10.25259/AUJMSR_49_2022

Umare, A., Seyoum, B., Gobena, T., Haile Mariyam, T., 2016. Hepatitis B virus infections and associated factors among pregnant women attending antenatal care clinic at Deder Hospital, Eastern Ethiopia. PloS one 11, e0166936.

Umer, A., Teklemariam, Z., Ayele, F., Mengesha, M.M., 2023. Prevalence of hepatitis B infection and its associated factors among pregnant mothers attending antenatal care at public hospitals at Hararghe, Eastern Ethiopia. Frontiers in Global Women's Health 4.

Wakjira, M., Darega, J., Oljira, H., Tura, M.R., 2022. Prevalence of hepatitis B virus and its associated factors among pregnant women attending antenatal care in Ambo town, Central Ethiopia: a cross-sectional study. Clinical Epidemiology and Global Health 15, 101054.

Wang, J.-S., Zhu, Q.-R., Wang, X.-H., 2003. Breastfeeding does not pose any additional risk of immunoprophylaxis failure on infants of hbv carrier mothers. International Journal of Clinical Practice 57, 100–102. https://doi.org/10.1111/j.1742-1241.2003.tb10435.x

Wiseman, E., Fraser, M.A., Holden, S., Glass, A., Kidson, B.L., Heron, L.G., Maley, M.W., Ayres, A., Locarnini, S.A., Levy, M.T., 2009. Perinatal transmission of hepatitis B virus: an Australian experience. Medical Journal of Australia 190, 489–492. https://doi.org/10.5694/j.1326-5377.2009.tb02524.x

Wondmeneh, T.G., Mekonnen, A.T., 2024. Epidemiology of hepatitis B virus infection among pregnant women in Africa: a systematic review and meta-analysis. BMC Infect Dis 24, 921. https://doi.org/10.1186/s12879-024-09839-3

WHO. Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021: accountability for the global health sector strategies 2016–2021: actions for impact. World Health Organization.

WHO. Guidelines on hepatitis B and C testing, 2017 https://www.who.int/publications/i/item/9789241549981 Accessed on April 23,2025

WHO. Hepatitis B, 2024a https://www.who.int/news-room/fact-sheets/detail/hepatitis-b Accessed on November 2, 2024.

WHO. Hepatitis B, 2022 https://www.who.int/news-room/fact-sheets/detail/hepatitis-b Accessed on November 5, 2024

WHO. Technical considerations and case definitions to improve surveillance for viral hepatitis: technical report. World Health Organization (WHO)., 2016 Accessed on March 26, 2025

WHO, 2020 Prevention of mother-to-child transmission of hepatitis B virus: guidelines on antiviral prophylaxis in pregnancy https://www.who.int/publications-detail-redirect/978-92-4-000270-8 Accessed on November 13, 2024

WHO. Hepatitia B vaccination coverage, 2024 https://immunizationdata.who.int/global/wiise-detail-page/hepatitis-b-vaccination-coverage?ANTIGEN=HEPB3&CODE=BFA. Accessed on Nov 15, 2024.

WHO. Immunization 2024 Ethiopia country profile. https://www.who.int/publications/m/item/immunization-2024-ethiopia-country-profile. Accessed on Nov 17, 2024

Yang, J., Zeng, X., Men, Y., Zhao, L., 2008. Elective caesarean section versus vaginal delivery for preventing mother to child transmission of hepatitis B virus – a systematic review. Virol J 5, 100. https://doi.org/10.1186/1743-422X-5-100

Yao, N., Fu, S., Wu, Y., Tian, Z., Feng, Y., Li, J., Luo, X., Yang, Y., Ji, F., Chen, Y., Liu, J., Zhao, Y., Chen, T., 2022a. Incidence of mother-to-child transmission of hepatitis B in relation to maternal peripartum antiviral prophylaxis: A systematic review and meta-analysis. Acta Obstetricia et Gynecologica Scandinavica 101, 1197–1206. https://doi.org/10.1111/aogs.14448

Yao, N., Fu, S., Wu, Y., Tian, Z., Feng, Y., Li, J., Luo, X., Yang, Y., Ji, F., Chen, Y., Liu, J., Zhao, Y., Chen, T., 2022b. Incidence of mother-to-child transmission of hepatitis B in relation to maternal peripartum antiviral prophylaxis: A systematic review and meta-analysis. Acta Obstet Gynecol Scand 101, 1197–1206. https://doi.org/10.1111/aogs.14448

Yazie, T.D., Tebeje, M.G., 2019. An updated systematic review and meta-analysis of the prevalence of hepatitis B virus in Ethiopia. BMC Infect Dis 19, 917. https://doi.org/10.1186/s12879-019-4486-1

Yi, P., Chen, R., Huang, Y., Zhou, R.-R., Fan, X.-G., 2016. Management of mother-to-child transmission of hepatitis B virus: Propositions and challenges. Journal of Clinical Virology 77, 32–39.

Zhang, L., Gui, X.-E., Wang, B., Fan, J.-Y., Cao, Q., Mullane, K., Liang, X.-L., 2016. Serological positive markers of hepatitis B virus in femoral venous blood or umbilical cord blood should not be evidence of in-utero infection among neonates. BMC Infect Dis 16, 408. https://doi.org/10.1186/s12879-016-1754-1

Zhang, S.-L., Yue, Y.-F., Bai, G.-Q., Shi, L., Jiang, H., 2004. Mechanism of intrauterine infection of hepatitis B virus. World J Gastroenterol 10, 437–438. https://doi.org/10.3748/wjg.v10.i3.437

Appendix A– Questionnaire English version

CROSS-SECTIONAL SURVEY ON PREVALENCE OF HEPATITIS B INFECTION, MTCT AND ASSOCIATED RISK FACTORS

General instruction- the questionnaire has five sections including questions regarding *socio-demographic characteristics, past medical history, social and behavioral risk factors, pregnancy and delivery information and mother to child transmission associated information.*

Section 1: Socio-Demographic Information
1. Age: ____ years
2. Age of infant:_____(in month)
3. Place of residence o Urban o Rural
4. Ethnic group o Nuer o Anuak o Other: _____
5. Religion o Orthodox o Muslim o Protestant o Other: _____
6. Education level o No formal education o Primary school o Secondary school o College/University
7. Occupation: o Employed o Private sector o House wife o Student
8. Marital status o Married o Single o Divorced o Widowed
9. Parity o Nulliparous o Multiparous
10. Household income: _____ ETB/month
Section 2: Past Medical History
1. History of blood transfusion? o Yes o No

2. History of any surgical procedures
    - Yes
    - No
3. History of dental procedures
    - Yes
    - No
4. History of abortion
    - Yes
    - No
5. History of other sexually transmitted infections (STIs)
    - Yes
    - No
6. Have you ever been diagnosed with a liver disease?
    - Yes
    - No
7. History of admission to a healthcare facility?
    - Yes
    - No
8. History of vaccination for Hepatitis B?
    - Yes
    - No

Section 3: Social and Behavioral Risk Factors

1. History of tattoos
    - Yes
    - No
2. History of body piercings
    - Yes
    - No
3. History of sharing sharp objects (knife, needle, razor, earring, blade)
    - Yes
    - No
4. History of traditional surgical practices. i.e genital mutilation, cauterization
    - Yes
    - No
5. Did you have traditional Uvelectomy/ tonsillectomy?
    - Yes
    - No
6. History of multiple sexual partners
    - Yes
    - No
7. Did any of your family members have HBV?
    - Yes
    - No
8. History of working in health facility
    - Yes
    - No

Section 4: Pregnancy and Delivery Information

1. Did you have regular ANC follow up?
    - Yes
    - No
2. Were you screened for HIV?
    - Yes(circle the result) P N Don't Know
    - No
3. Mode of delivery?
    - Vaginal delivery
    - Instrumental Delivery
    - Cesarean Section Delivery
4. Any complication during pregnancy, labor and delivery?
    - Yes, Specify_____
    - NO
5. Place of previous delivery
    - Home
    - Health facility
    - Both

Section 5. Mother To Child Transmission Information

1. Were you screened for HBV during your last pregnancy? (if the answer is " don't know or No, jump to question 5)?
    - Yes
    - No
    - Don't know
2. If yes, what was the result?
    - positive
    - Negative
3. If positive, did you receive antiviral treatment?
    - Yes
    - No
4. Did your infant receive any MTCT preventive methods?
    - HepB-BD
    - HBIG
    - Both
    - None
5. Were additional doses of the Hepatitis B vaccine given to your infant?
    - Yes
    - No
6. Did you breast feed?
    - Yes
    - No
7. Had you previously given birth?
    - Yes
    - No
8. If yes, were any of your children diagnosed with HBV?
    - Yes
    - No

THANK YOU FOR YOUR COOPERATION!

Appendix B- Questionnaire Amharic version

የሄፐታይተስ ቢ ኢንፌክሽን፣ ከእናት ወደ ልጅ መተላለፍ እና ተያያዥ ኢጋላጭ መንስኤዎች ሰርጭት ላይ የሚደረግ ዳሰሳ ኣጠቃላይ መመሪያ- መጠይቁ ማህበረሰባዊ፣ሥኑ-ሕዝብ ባህሪያት፣ ያለፈው የሕክምና ታሪክ፣ ኢጋላጭ ማህበራዊ እና የግል ባህሪዎ፣ የእርግዝና እና የወሊድ መረጃ እና ከእናት ወደ ልጅ መተላለፍ ጋር ተያያዥ መረጃዎችን የሚመለከቱ ጥያቄዎችን ጨምሮ አምስት ክፍሎች አሉት

ክፍል 1: *ማህበረሰባዊ-ሰነ-ሕዝባዊ መረጃ*
11. ዕድሜ: _____ ዓመት
12. የጨቅላ ህጻን ዕድሜ፡_____(በወር)
13. የመኖሪያ ቦታ o ከተማ o ገጠር
14. ዘር o ኑዌር o ኣኙዋክ o ሌላ፡ _____
15. ሃይማኖት o ኦርቶዶክስ o ሙስሊም o ፕሮቴስታንት o ሌላ፡ _____
16. የትምህርት ደረጃ o መደበኛ ትምህርት የለም o የመጀመሪያ ደረጃ ትምህርት ቤት o ሁለተኛ ደረጃ ትምህርት ቤት o ኮሌጅ/ዩኒቨርስቲ
17. ሥራ o ተቀጠረ o የግል ዘርፍ o የቤት እመቤት o ተማሪ
18. የጋብቻ ሁኔታ o ያገባች o ያላገባች o የተፋታች o ባሏ የሞተባት
19. የወሊድ ብዛት o አንድ o ከአንድ በላይ
10. የቤተሰብ ገቢ፡- _____ (በወር)
ክፍል 2፡ የህክምና ታሪክ
ነ. ደም ተለግሰሽ ታውቂያለሽ? o አዎ o አይ
2. ማንኛውም የቀዶ ጥገና ሂደቶች አድርገሽ ታውቂያለሽ? o አዎ o አይ

3. የጥርስ ህክምና ሂደቶች አድርገሽ ታውቂያለሽ? o አዎ o አይ
4. የፅንስ መውረድ አጋጥሞሽ ያውቃል? o አዎ o አይ
5. ሌሎች በግብረ ሥጋ ግንኙነት የሚተላለፉ ኢንፌክሽኖች (STIs) አጋጥሞሽ ያውቃል? o አዎ o አይ
6. በህክምና የጉበት በሽታ ተብለህ ታውቃለሽ? o አዎ o አይ
7. ጤና ተቋም ተኝተሽ ታክመሽ ታውቃለሽ? o አዎ o አይ
8. ለሄፐታይተስ ቢ ክትባት ወስደሽ ታውቃለሽ? o አዎ o አይ
ክፍል 3: *አጋላጭ ማህበራዊ እና የግል ባህሪያት*
1. ንቅሳት አለሽ? o አዎ o አይ
2. የሰውነት መበሳት አድርገሽ ታውቃለሽ? o አዎ o አይ
3. ስለታም ነገሮች የመጋራት ባህል አለሽ? (ቢላዋ፣ መርፌ፣ ምላጭ፣ ጉትቻ፣ ምላጭ) o አዎ o አይ
4. የባህላዊ ቀዶ ህክምና ተደርጎልሽ ያውቃል? (የሴት ልጅ ግርዛት፣ ቆዳን በጋለ ብረት መጥነስ) o አዎ o አይ
5. ባህላዊ እንጥል/ ቶንሲል መቁረጥ ተደርጎልሻል? o አዎ o አይ
6. ከአንድ በላይ ወሲባዊ አጋር አለሽ? o አዎ o አይ
7. ከቤተሰብዎ አባላት መካከል በኤች.ቢ.ቪ. የተጠቃ አለ? o አዎ o አይ
8. በጤና ተቋም ውስጥ ሰርተሻል? o አዎ o አይ
ክፍል 4፡ የእርግዝና እና የወሊድ መረጃ
1. መደበኛ የእርግዝና ክትትል ነበረሽ? o አዎ o አይ

2. ለኤችአይቪ ተመርምረሻል? o አዎ (ውጤቱ አክብብ) P N አላውቅም o አይ
3. የወሊድ ሁኔታ? o በማህጸን o በመሳሪያ የታጋዘ o በቀዶ ህክምና
4. በእርግዝና፣ በምጥ እና በወሊድ ጊዜ ያጋጠመ ችግር ነበር? o አዎ፣ ይግለጹ_____ o አይ
5. የቀድሞ የወሊድ ስፍራ o ቤት o የጤና ተቋም o ሁለቱም
ክፍል 5. ከእናት ወደ ልጅ መተላለፍ ጋር የተያያዙ መረጃዎች
1. ባለፈው እርግዝናዎ ወቅት ለኤች.ቢ.ቪ ምርመራ ተደርጎልሻል? (መልሱ “አላውቅም ወይም የለም ከሆነ ወደ ጥያቄ 5 ይዝለሉ)? o አዎ o አይ o አላውቅም
2. መልሱ አዎ ከሆነ፤ ውጤቱ ምን ነበር? o አለ (positive) o የለም (negative)
3. ውጤቱ አለ ከነበር የፀረ-ቫይረስ ህክምና ወስደሻል? o አዎ o አይ
4. ልጅዎ ከእናት ወደ ልጅ እንዳይተላለፍ መከላከያ አማራጭ ተሰጥቶል? o HepB-BD o HBIG o ሁለቱም o ምንም
5. ልጅዎ የሄፐታይተስ ቢ ክትባት ወስደዋል? o አዎ o አይ
6. ጡት አጥብተሻል? o አዎ o አይ
7. ከዚህ ወሊድ በፊት ልጅ አለሽ? o አዎ o አይ
8. አዎ ከሆነ፣ በኤች.ቢ.ቪ የታመመ ልጅ ነበር? o አዎ o አይ

ስለ ትብብርዎ እናመሰግናለን!

Appendix C- Questionnaire Nuer Version

THƆRBƐY MI RƆR-THƐKCIƆNAL KUI̱ PREBALƐNTH DUIƆ ƔƐPATITH B INFEKCIƆN, MTCT KƐN ATHTHÖCIƐTED RISK PAKTƆRI̱

Ɔiic kɛɛliw- ɛn thiecni̱ tekɛ gua̱th daŋ dhie̱ec ti̱ cu määnı̱ thiecni̱ ti̱ lot rɔ kɛ kui̱ ciɛŋä nath ciɛŋ demogra̱pik, lëëŋ wal tëë wal, ciɛŋ nath kɛnɛ tin dee naath ya̱r, ruët kɛnɛ ruac kɛ kuic dapä kɛnɛ ruac mi thiäk kɛ kuic man gatdä.

Kä 1: Thöciö-Demögra̱pik Inpɔrmɛciɔn
1. Ruɔ̱n: ____ run 2. Run gatdä:_____(kɛ pay)
3. Gua̱a̱th ciɛŋä o Rëëk o Rural
4. Buɔ̱n dööri o Nuëri o Anuak o Kɔ̱kiɛn:
5. Kuth o Ɔrthödɔk o Muthlimni̱ o Prötɛthtan o Kɔ̱kiɛn:
6. Lebel duel gɔärä o Thiɛlɛ duel gɔärä mi thuɔ̱k o Duel gɔärä in nhiam o Duel gɔärä in rɛwdɛ o Kɔllej/Yuni̱berthi̱ti̱
7. La̱tdɛ: o Cakɛ lät o Praibɛt thɛktɛr o Ciek duëël o Gat duëlgɔ̱rä
8. Ciaŋ kuën o Ci̱ kuɛɛn o Kɛl o Ci̱kɛ da̱k o ciek mi ci li̱w
9. Pari̱ti̱ o Nulli̱paröth o Multi̱parɔth
10. Yiɔ̱w tin nöönkɛ ɛ ji̱ dhɔar: _____ ETB/pay
Kä 2: Ɣi̱thtöri̱ Medikal ëë wal
1. Lɛ̈ëŋ tin ca lat kɛ kui̱ riɛm? o Ɣɔɔ̱n o /Ciɛ jɛn
2. Ɣi̱thtöri̱ duŋ ɛni̱ thɛrgi̱kal pröciɛri̱ o Ɣɔɔ̱n

- /Ciɛ jɛn

3. Ɣi̱thtöri̱ duŋ dental pröciɛri̱
   - Ɣɔ̱ɔ̱n
   - /Ciɛ jɛn

4. Ɣi̱thtöri̱ duŋ kä ɣöö ba gat ka̱m raar
   - Ɣɔ̱ɔ̱n
   - /Ciɛ jɛn

5. Ɣi̱thtöri̱ jua̱thni̱ kɔkiɛn tin la nööŋkɛ ɛ tɔ̱ɔ̱c (STIs)
   - Ɣɔ̱ɔ̱n
   - /Ciɛ jɛn

6. Ci ji̱n tekɛ juey mi ci jiäk kä ji̱?
   - Ɣɔ̱ɔ̱n
   - /Ciɛ jɛn

7. History kɛ kui̱ kä ɣöö ca ji̱ naŋ duelwa̱l?
   - Ɣɔ̱ɔ̱n
   - /Ciɛ jɛn

8. Ɣi̱thtöri̱ duŋ wäl tumä kɛ kui̱ Ɣɛpai̱tai̱th B?
   - Ɣɔ̱ɔ̱n
   - /Ciɛ jɛn

Kä 3: Thɔciɔl kɛnɛ Biɛbiöral Ri̱thki̱ Paktɔri̱

1. Ɣi̱thtöri̱ duŋ tattooni̱
   - Ɣɔ̱ɔ̱n
   - /Ciɛ jɛn

2. Ɣi̱thtöri̱ duŋ pi̱ɛɛ̱rni̱ pua̱ny
   - Ɣɔ̱ɔ̱n
   - /Ciɛ jɛn

3. Ɣi̱thtöri̱ duŋ nyuak ŋɔaani̱ ti̱ thɛp (thɛp, ri̱ääp, rɛthɛr, ji̱th, thɛp)
   - Ɣɔ̱ɔ̱n
   - /Ciɛ jɛn

4. Ɣi̱thtöri̱ duŋ ciɛŋni̱ thɛrgi̱kalä tëë wal. c.e. ji̱nital muti̱lɛciɔn, kauteraidhɛciɔn
   - Ɣɔ̱ɔ̱n
   - /Ciɛ jɛn

5. Ci ji̱n ci Ubɛlektömi̱/ tɔnthi̱lɛktömi̱ la̱t kɛ ciaaŋ?
   - Ɣɔ̱ɔ̱n
   - /Ciɛ jɛn

6. History kä nɛy ti ŋuan ti ci rɔ̱ mat kɛ kɛ
   - Ɣɔ̱ɔ̱n
   - /Ciɛ jɛn

7. Te ram kɛl kä ji dhɔaru mi tekɛ juey HBV?
   - Ɣɔ̱ɔ̱n
   - /Ciɛ jɛn

8. Lëëŋ lätni̱ rɛy gua̱th puɔlä pua̱a̱ny
   - Ɣɔ̱ɔ̱n
   - /Ciɛ jɛn

Kä 4: Ruac kɛ kui̱ dapä kɛnɛ dap

1. Ci ANC tekɛ guur ni ciaŋ?
   - Ɣɔ̲ɔ̲n
   - /Ciɛ jɛn

2. Ci ji̲n ca ji̲ thɛm kɛ kui̲ HIV?
   - Ɛɛ(circle min ci̲ ben raar) P N /Cä jɛ ŋäc
   - /Ciɛ jɛn

3. Duɔ̲ɔ̲p in dëë lätni̲ jɛ?
   - Vaginal deli̲beri̲
   - Ini̲thti̲rumɛntal Deli̲beri̲
   - Thɛthɛrian Thɛkcin Deli̲beri̲

4. Te kɛ riɛk mi ci tuɔk kɛ gua̲th dapä, gua̲th dapä kɛnɛ gua̲th dapä?
   - Ɣɔ̲ɔ̲n, Latdɛ
   - /Ciɛ jɛn

5. Gua̲a̲th ëë ca la̲th thi̲n ni̲ wal
   - Ciëŋ
   - Gua̲a̲th puɔlä pua̲a̲ny
   - Kɛn da̲ŋ rɛw

Kä 5. Ruac kɛ kui̲ gatä kä man kɛnɛ gatdɛ

1. Ci ji̲n ca ji̲ thɛm kɛ kui̲ HBV kɛ gua̲a̲th ëë ci̲ ji̲n tekɛ gatdu min jɔak? (mi ci luɔcdɛ ɛ " /cä jɛ ŋäc kiɛ . /Ciɛ jɛn, jäl kä thiec 5)?
   - Ɣɔ̲ɔ̲n
   - /Ciɛ jɛn
   - /Cä jɛ ŋäc

2. Mi ɛ thuɔ̲k, ɛŋu mi ci tuɔɔk?
   - puɔ̲thi̲tib
   - Nɛgɛtib

3. Mi ci jiäk, ci wal ti jiäk jek?
   - Ɣɔ̲ɔ̲n
   - /Ciɛ jɛn

4. Ci gatdu min tɔt tekɛ dup ti gaŋ jɛ kä MTCT?
   - ƔɛpB-BD
   - HBIG
   - Kɛn da̲ŋ rɛw
   - Thiɛlɛ

5. Ci wal ti kɔ̲ŋ ti ca thöp kɛ kui̲ Hepatitis B thöp gatdu?
   - Ɣɔ̲ɔ̲n
   - /Ciɛ jɛn

6. Kä ji̲n ci thɔ̲l thöp?
   - Ɣɔ̲ɔ̲n
   - /Ciɛ jɛn

7. Ci ji̲n ci gat kɔn dap?
   - Ɣɔ̲ɔ̲n
   - /Ciɛ jɛn

8. Mi ɛ thuɔ̲k, te gatdu mi ca jek kɛ juey HBV?
   - Ɣɔ̲ɔ̲n
   - /Ciɛ jɛn

Appendix D- Questionnaire Anuak version

Kwäänø bäät Lwaak kiper Nut mar Täw Cwïny B, Muuö ki bang Mïïö ni Cøøa bang Nyilaal ki Jammi møøk mo Gääbö mo Reyyø

Jöör Tïïc Bäre: Pïëc coong luup adïëri mooi käädö ki thängngi abïïc ni guta pïëc mo gääbö ki bëët gääbö-kwään jiy, leerø mar luum jööt dëël, jammi moo raany bëët dwätö ki yi bëëtö, jäc ki luup lwaar ki luup mo gääbö ki muuö ki bang mïïö ni cøøa bang nyilaal.

Thäängö 1: Luum Bëët Gääbö-Kwään Jiy
1. Cwiiri: Cwiiri ma_____
2. Cwiiri mo Nyilaal:_____(ni kwaan ka dwädi)
3.kar bëëtö o Pääny o Teegø
4. Wï jur: o Nwäär o Anywaa o Møøk:_____
5. Jwøk man Lam: o Ørthødøk o Muucilïm o Pørøtectant o Møøk:_____
6. Ogat Göör o Bäng Göör mo ree ee koorø o Ogat göör mana dikwøng o Riet ogat göör o Køølec/Yuunibööciti
7. Tïïc: o Cere da tïïc o Tïïc mare keere o Dhaang paac o Nyilaar göör
8.Lïng bëët dhak/cwøw o Onywømø o Ker nywømø o Geno pääö o Cï thøø(Cwøre othøw)
9. Kwör lwaar o Yie aciel o Olwaar kwöre mo thööth
10. Jammi mo joot Jø tuung yi kal:Bïrï mo Ithoopia ma_____/dwääy
Thäängö 2: Leerø mo opöödhö kiper øt-jaath
1. Leerø mar kïth remø yi dëël o Yaa o Bänggø
2. Leerø mar jap rëët o Yaa

- Bänggø

3.Leerø mar luum lak
- Yaa
- Bänggø

4. Leerø mar mänh wøk
- Yaa
- Bänggø

5. Leerø mar täwë møøk mo muuö ki køør gääp dhaagø ki dicwøø
- Yaa
- Bänggø

6. Da täw cwïny mo ongiiø riï?
- Yaa
- Bänggø

7. Leerø mar kïth kar tïï jööt dëël
- Yaa
- Bänggø

8. Leerø mar maath jaath mar raany täw cwïny B
- Yaa
- Bänggø

Thäängö 3: Gïïa reyyø kiper Bëët gäääbö ki yi bëëtö

1.Leerø mar käde
- Yaa
- Bänggø

2.Leerø mar cööp dëël
- Yaa
- Bänggø

3. Leerø mar nywaak jammi(cakïïn, lïlmuw, muuc,gwet-ïdhï)
- Yaaa
- Bängø

4. Leerø mar rëët/ngøl ki køør kööngngö,i.e ngøl dëër dhaagø/dëër dicwøø,waang-atwøda
- Yaa
- Bänggø

5.Gwëëni marï okäl wøk ki køør kööngngö?
- Yaa
- Bänggø

6. Leerø mar niine/ngøøth ki jiy mo thööth
- Yaa
- Bänggø

7. Da twöngö/virus mar täw cwïny B mo ongiiø re ngat tuung yi kal marï?
- Yaa
- Bänggø

8.Leerø mar tïïc kar tïï jööt dëël
- Yaa
- Bänggø

Thäängö 4: Luum Jäc ki Lwaar

1. Da luum nyööt/lwaar mo køøre yiï løppø?
- Yaa
- Bänggø

2. Twöng/täw ËËDC/ HIV ongiiø dëërï? o Yaa o Bänggø o Kuua
3. Jöör lwaar o Lwaar ki mur o Lwaar ki jammi mo rëët o Maath këëmikal mar cicäm
4. Da rääm/gïn mo leth re jäc, lwaar ki mäc? o Yaa, Jierï_____ o Bänggø
5. Kar lwaar mana dikwøng o Paac o Øt-jaath o Ariew moøgø bëët
Thäängö 5: Luum Muuö ki bang Mïïö ni cøøa bang Nyilaal(MTCT)
1. Twöng ËËDC/HIV angec riï re lwaar marï mana näk anguudi?(Ninäk løk-pïëc bee 'Kuua' wala 'Bänggø', päär bang pïëc 5). o Yaa o Bänggø o Kuua
2. Ninäk løk-pïëc bee Yaa, a gïne na joot? o Nut mar ËËDC/positive o Bäng ËËDC/negative
3. Ninäk täw ËËDC ojoodø yi remø marï, da jet raany baairec mo ocïbö jïrï? o Yaa o Bänggø
4. Nyilaal marï da jöör mänö mar muuö ki bang mïïö ni cøøa bang nyilaal mo ee joodø? o Täw cwïny B-BD o Täw cwïny BIG o Moøgø bëët o Bänggø
5. Da döödi møøk mo jinni mo cööp kiper täw cwïny B mo ocïbø jï nyilaal marï? o Yaa o Bänggø
6. Yïïno dhwödhö? o Yaa o Bänggø
7. Yïïno lwaar dikwøng? o Yaa o Bänggø
8. Ninäk løk-pïëc bee Yaa, da Nyilaal marï mo twöng täw cwïny B ongiiø dëëre? o Yaa o Bänggø

Yïïna Pwøa kiper køny marï

Appendix E– Informed consent English version

Information sheet

Introduction- Hello, my name is Bethelhem Teshome. I am 2nd year masters student at Aklilu Lemma Institute of Pathobiology. I am conducting a research on hepatitis B which is a common health problem in Gambella. This informed consent form is for mothers attending EPI clinic to have their infants aged between 9-12 months vaccinated and who I'm inviting to participate in the research. The title of the research is "the prevalence of Hepatitis B Virus Infection, mother-to-child transmission and associated risk factors among mothers attending Gambella health facilities, South-Western Ethiopia".

The **aim** of this study is to assess the burden of hepatitis B virus infection, mother to child transmission and identify factors contributing to both HBV infection and mother to child transmission among mothers. This will provide insights for designing effective preventive strategies.

Study process-If you are willing to participate, you will be asked few questions via interview lasting approximately 15-20 min. your medical history will be reviewed. Then small blood sample (5 ml) will be collected for testing the presence of Hepatitis B virus. If the result showed the presence of the virus, other two tests will proceed to help us understand more on the risk of mother to child transmission. Subsequently with your assent, small blood sample (2-3ml) will be collected by heel prick from your infant to test for the presence of hepatitis B if your result turns positive.

Risk and benefits- There is minimal risks involved with the blood sample collection, including slight pain, bruising, or discomfort. There is no direct financial benefits for participation. Your involvement is greatly helpful for better understanding and management of HBV in mothers and infants in your community. If HBV is detected, you will be linked to appropriate medical care in the facility.

Voluntary participation and rights- All the information you provide will remain confidential. Your name or any other identifying information will not appear in any reports or publications. Data will be stored securely and accessed only by the research team. Your participation is entirely voluntary. You may choose not to participate or withdraw from the study at any time without any impact on your medical service you receive.

If you have any question about the study or would love to be informed of the results, you can contact the principal investigator.

Name- Bethelhem Teshome

Phone number- +251943504172/ +251910475705 Email- teshebeti2016@gmail.com

Consent form

Are you willing to participate in this study?

- Yes……..Continue to next part
- No……thank the respondent and stop here

I have read or had the information sheet read and explained to me in a language I understand. I know that my participation is voluntary and can withdraw at any time without any consequence. I have had the opportunity to ask questions, and they have been answered efficiently.

Participant's signature- _____ Date- _____

Principal investigator's signature–_____ Date-_____

Thank you for your cooperation!

Assent form

Are you willing to participate in this study?

- Yes……..Continue to next part
- No……thank the respondent and stop here

I have read or had the information sheet read and explained to me about this study. I have had the opportunity to ask questions and receive satisfactory answers. I understand that participation is voluntary, and can withdraw my child from the study at any time. I understand that my child's participation involves the collection of small blood sample for the test and provide relevant health information. I am aware that all information will remain confidential.

Parent's signature- _____ Date- _____

Principal investigator's signature-_____ Date-_____

Thank you for your cooperation!

Appendix F- Informed consent form Amharic version

ክፍል 1- የመረጃ ቅፅ

መግቢያ- ሰላም ቤተልሄም ተሾመ እባላለሁ። በአክሊሉ ለማ የፓቶባዮሎጂ ተቋም የ2ኛ ዓመት የማስተርስ ተማሪ ነኝ። በጋምቤላ የተለመደ የጤና ችግር በሆነው ሄፓታይተስ ቢ ላይ ጥናት እያደረግሁ ነው። ይህ በመረጃ ላይ የተመሰረተ የስምምነት ቅጽ ከ 9-12 ወር ያሉ ልጆቻቸውን መደበኛ ክትባት ለማስከተብ የሚመጡ እናቶችን ወደ ጥናት ለመጋበዝ የተዘጋጀ ነው። የምርምሩ ርዕስ "በጋምቤላ ከተማ በሚገኙ ጤና ተቋማት በሚመጡ እናቶች ላይ የሄፕታይተስ ቢ ቫይረስ ስርጭት፣ ከእናት ወደ ልጅ መተላለፍ እና ተያያዥ አጋላጭ ሁኔታዎች " የሚል ነው።

የዚህ ጥናት **ዓላማ** የሄፐታይተስ ቢ ቫይረስ ኢንፌክሽን፣ ከእናት ወደ ልጅ መተላለፍ ያለውን ጫና ለመገምገም እና ለኤች.ቢ.ቪ ኢንፌክሽን እና ከእናት ወደ ልጅ እንዲተላለፉ አስተዋጽኦ የሚያደርጉ ሁኔታዎችን ለመለየት ነው። ይህ ውጤታማ የመከላከያ ስልቶችን ለመንደፍ አቅጣጫን ይሰጣል።

የጥናቱ ሂደት- ለመሳተፍ ፈቃደኛ ከሆኑ ፣ ከ15-20 ደቂቃ የሚቆይ ጥቂት ጥያቄዎች በቃለ መጠየቅ ይጠየቃሉ። የሕክምና ታሪክዎ ይታያል ። ከዚያም የሄፐታይተስ ቢ ቫይረስ መኖሩን ለመመርመር ትንሽ የደም ናሙና (5 ሚ.ሊ) ይወሰዳል ። ውጤቱ የቫይረሱን መኖር ካሳየ ከእናት ወደ ልጅ የመተላለፍን ዕድል የበለጠ ለመረዳት እንድንችል ሌሎች ሁለት ምርመራዎች ይወሰዳል ። በመቀጠልም የልጅዎን ሁኔታ ለማወቅ በእርስዎ ስምምነት ትንሽ የደም ናሙና (2-3ml) ከጨቅላዎ ተረከዝ ይሰበሰባል።

ጥቅምና ጉዳቱ-ከደም ናሙና አወሳሰድ ጋር ተያይዞ በጣም አነስተኛ ጉዳት ሊያጋጥም ይችላል። ይህም ትንሽ ህመም፣ የቆዳ መቆጣት ወይም ምቾት ማጣት ናቸው። ለተሳትፎ ቀጥተኛ የገንዘብ ጥቅማጥቅሞች የሉም። የእርስዎ ተሳትፎ በማህበረሰብዎ ውስጥ በእናቶች እና ጨቅላ ህጻናት ላይ ያለውን የኤች.ቢ.ቪ. ኤች.ቢ.ቪ ስርጭትና ቁጥጥር ለመረዳት ይረዳል። እንዲሁም ቫይረሱ ከተገኘ፣ በተቋሙ ውስጥ ካለው ተገቢ የህክምና አገልግሎት ጋር ይገናኛሉ።

በፈቃደኝነት ላይ የተመሰረተ ተሳትፎ እና መብቶች - ሁሉም የሚያቀርቡት መረጃ ሚስጥራዊ ሆኖ ይቆያል። ስም ወይም ሌላ መለያ መረጃ በማንኛውም ሪፖርቶች ወይም ህትመቶች ላይ አይታይም። መረጃው ደህንነቱ በተጠበቀ ሁኔታ ይከማቻል፤ በተመራማሪው ቡድን እጅ ብቻ ይሆናል። የእርስዎ ተሳትፎ ሙሉ በሙሉ በፈቃደኝነት ነው። በሚቀበሉት የህክምና አገልግሎት ላይ ምንም አይነት ተጽእኖ ሳይኖር በማንኛውም ጊዜ ላለመሳተፍ ወይም በጥናቱ ላለመሳተፍ መምረጥ ይችላሉ። ስለ ጥናቱ ምንም አይነት ጥያቄ ካሎት ወይም ስለ ውጤቶቹ እንዲነገርዎት ከፈለጉ ዋናውን መርማሪ ማነጋገር ይችላሉ።

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ክፍል 2- የስምምነት ቅጽ

በዚህ ጥናት ለመሳተፍ ፈቃደኛ ነዎት?

- አዎ ……ወደ ቀጣዩ ክፍል ይቀጥሉ
- አይ ……ምላሽ ሰጪውን አመስግኑ እና እዚህ ያቁሙ

እኔ በምረዳው ቋንቋ የመረጃ ወረቀቱን አንብቤ ወይም ተነቦልኝ በሚገባ ተረድቻለሁ። የእኔ ተሳትፎ በፈቃደኝነት እንደሆነ እና በማንኛውም ጊዜ ያለምንም ችግር ማቋረጥ እንደምችል አውቃለሁ። ጥያቄዎችን ለመጠየቅ እድሉን አግኝቻለሁ፤ እነሱም በሚገባ ምላሽ ተሰቶኛል።

የተሳታፊ ፊርማ - _____ ቀን - _____

የዋና መርማሪ ፊርማ-_____ ቀን-_____

ስለ ትብብርዎ እናመሰግናለን!

ክፍል 3- የፍቃድ ቅጽ

በዚህ ጥናት ለመሳተፍ ፈቃደኛ ነዎት?

- አዎ ……ወደ ቀጣዩ ክፍል ይቀጥሉ
- አይ ……ምላሽ ሰጪውን አመስግኑ እና እዚህ ያቁሙ

በዚህ ጥናት ላይ የመረጃ ወረቀቱን አንብቤ ወይም ተነቦልኝ በሚገባ ተረድቻለሁ። ጥያቄዎችን ለመጠየቅ እና አጥጋቢ መልሶችን ለመቀበል እድሉን አግኝቻለሁ። ተሳትፎ በፈቃደኝነት እንደሆነ እና ልጄን በማንኛውም ጊዜ ከጥናቱ ማውጣት እንደምችል ተነግሮኛል። ሁሉም መረጃዎች በሚስጥር እንደሚቆዩ አውቃለሁ።

የወላጅ ፊርማ - _____ ቀን - _____

የዋና መርማሪ ፊርማ-_____ ቀን-_____

ስለ ትብብርዎ እናመሰግናለን!

Appendix G –Informed Consent Nuer version

Wargak ruac

Nyuuth- Ɣɔ̲ɔ̲n, ciötdä ɛ Beth-ɛl-ɣɛm Tethhöme. Ɣän ɛ gat duël gɔ̲rä mi̲ te rɛy runä in rɛwdɛ kä Aklilu Lemma Ini̲thti̲tut duŋ Pathöbaölöji̲. Ɣän la̲tdä ri̲thääc kɛ kui̲ ɣe̲pati̲th B min la riɛk puɔlä pua̲a̲ny mi̲ la tuɔɔk kä Gambella. Ɛn wargak ɛmɛ ɛ ruac kɛ kui̲ man gatä tin wä kä EPI ki̲li̲nik kɛ ɣöö ba gaatkiɛn tin te kam päthni̲ da̲ŋ 9-12 wäl tumä wäl kä nɛy tin cä cɔl i̲ bi̲ kɛ ben te rɛy ri̲thäächä. Kä ci̲öt ri̲thäächä ɛ "kä ta̲a Ɣe̲pai̲tai̲th B Bai̲ra̲th Inpɛkciɔn, ja̲l man-kɛ-gatdɛ kɛnɛ tin thi̲ääk kɛ riɛk rɛy man gatä min wä kä Gambella gua̲th puɔlä pua̲a̲ny, Thɔth-Wɛthɛrɛn I̲thi̲öpia".

Kä luɔt ŋi̲i̲cä ɛmɛ ɛ ɣöö ba riɛk ɣe̲pai̲tai̲th B bai̲ra̲th inpɛkciɔn guic, min naŋkɛ ɛ man gatdä kɛnɛ ŋi̲e̲c kä tin no̲o̲ŋ juey HBV kɛnɛ min naŋkɛ ɛ man gatdä rɛy man gatdä min dapkɛ. Nɛmɛ bɛ naath moc kɛ ŋäc mi̲ dëë lätni̲ ca̲a̲p tin dëë gaŋ ni̲ jua̲th.

La̲t ŋi̲i̲cä-Mi go̲ri ɣöö bi te rɛy ŋi̲i̲cä, ba ji thiec kɛ thiecni ti tɔt kɛ rɛy ruac mi bi naŋ kɛ minitni 15-20. ba lëëŋku tin ca gɔ̲r piny kɛ kui̲ wal nyɔk kɛ di̲l guec. Kɛ kɔrɛ ba riɛm mi̲ tɔt (5 ml) ka̲n kɛ ɣöö ba thɛm i̲ te Ɣepati̲th B bai̲ra̲th thi̲n. Mi ci min ca jiek nyoth ɛn ɣöö te juey ɛmɔ thi̲n, bi thëm da̲ŋ rɛw kɔ̲kiɛn wä nhiam kɛ ɣöö ba kɔn luäk kɛ ŋäc mi di̲i̲t kɛ kui̲ rikä min de nöö̲ŋ ɛ man gatdä. Kɛ kɔrɛ kɛ nhökdu, ba riɛm mi tɔt (2-3ml) ka̲m raar kɛ thëm gɔ̲ɔ̲kä gatdu kɛ ɣöö ba thɛm i̲ tekɛ ɣe̲pai̲taith B mi̲ ci̲ ben raar ɛ la puɔ̲thi̲tip.

Risk kɛnɛ min de jek- Tekɛ riɛk mi tɔt mi de tuɔk kɛ riɛm, amäni bɛc mi tɔt, ŋëth, kiɛ mi jiäk. Thiɛlɛ yiöw ti de ji jek mi ci rɔ mat thin. Ɛn ɣöö bi rɔ mat thin bɛ ji luäk ɛlɔ̲ŋ kɛ ŋäc mi gɔa kɛnɛ tiet HBV kä man gatä kɛnɛ gaat tin tɔatni rɛy wecdu. Mi ca HBV jek, ba ji mat kä duel waal mi ro̲ŋ rɛy duel waal.

Kä mat nath kɛ lɔcdu kɛnɛ cuŋ- Ruacni diaal tin ci lat bi kɛ te thin. Ciötdu kiɛ ruac in dɔ̲ŋ in nyooth ɣöö ɛ ji̲n /ca jɛ bi la̲th rɛy ripɔ̲tni̲ kiɛ warɛgakni̲ tin ca gɔ̲r. Data ba kɛ tɔ̲w ɛ gɔaa kä ba kɛ jek ɛ buɔ̲n ri̲thäächä kärɔa. Kä matdu rɛydɛ kɛɛliw ɛ mi̲ la̲tkɛ kɛ lɔcdu. Ji̲n deri kuany ɛn ɣöö /ci bi mat kiɛ bi rɔ woc rɛy ŋi̲i̲cä kɛ gua̲a̲th mi ci wä mi thiɛl mi ci rɔ gɛr kä lät wal tin ci jek.

Mi te kɛ thiec kɛ kuic ŋi̲i̲cä kiɛ go̲ri ɣöö ba ji la̲r min ca jek, deri ruac kɛ kuär in di̲i̲t.

Ciöt- Beth-ɛl-ɣɛm Teth-ɣɔm

Nämbär tɛlɛpoonä- +251943504172/ +251910475705

Imeɛl- tethɛbeti2016@gmail.kɔm

Puɔm nhök

Jin nhɔki jɛ ɛn ɣöö bi rɔ mat rɛy ŋjiicä ɛmɛ?

o Ɛɛ.....Guɔrɛ jɛ kä min dɔŋ

o /Ciɛ jɛn.....lar ram min ci thiec tëthlɔaac kä cuɔŋ ɛn wanɛ

Ɣän cä kuɛn kiɛ cä wargak ruac kuɛn kä ca lat kä ɣä kɛ thok mi ŋacä. Ɣän ŋacä jɛ ɛn ɣöö matdä rɛydä ɛ mi latkɛ kɛ lɔcdä kä dëë rɔ woc guaathni diaal ɛ thiɛl mi dëë nööŋ. Ɣän cä guaath jek kɛ ɣöö bä thiecni thiec, kä ca kɛ loc a gɔaa.

Ciaŋ ram min te rɛydɛ- _____ Cäŋ-_____

Thignature duŋ Principal Inbɛthtɛtɔr–_____ Cäŋ-_____

Ɣän tɛth lɔcdä kɛ kui lätnikun kɛɛl!

Puɔm nhök

Jin nhɔki jɛ ɛn ɣöö bi rɔ mat rɛy ŋjiicä ɛmɛ?

o Ɛɛ.....Guɔrɛ jɛ kä min dɔŋ

o /Ciɛ jɛn.....lar ram min ci thiec tëthlɔaac kä cuɔŋ ɛn wanɛ

Ɣän cä kuɛn kiɛ cä warɛgak in ca gɔr kuɛn kä ca lat kä ɣä kɛ kui ŋjiicä ɛmɛ. Ɣän cä guaath jek kɛ ɣöö bä thiecni thiec kä cua luɔc thieecni ti gɔw jek. Ɣän ŋacä jɛ ɛn ɣöö mat nath rɛy ŋjiicä ɛ mi latkɛ kɛ lɔcdɛ, kä derɛ gatdä woc rɛy ŋjiicä ɛni guaath. Ɣän ŋacä jɛ ɛn ɣöö mat gatdä thin matdɛ ni ɣöö ba riɛm mi tɔt kam raar kɛ kui thëm kɛnɛ ŋun ruaacni tin lot rɔ kɛ kui puɔlä puaany. Ɣän ŋacä jɛ ɛn ɣöö ruaacni diaal bikɛ te thin ɛ la ti ca tëë.

Ciaŋ guan kɛnɛ man-_____ Cäŋ-_____

Thignature duŋ Principal Inbɛthtɛtɔr-_____ Cäŋ-_____

Ɣän tɛth lɔcdä kɛ kui lätnikun kɛɛl!

Appendix H- Informed Consent Anuak Version

Warkan Luup mo Coong

Bwödhi: Dëëtu jööt, nyengnga Bethelem Thecoome. A ena riet cwiiri mar riet dïgïrïï Kar Göör mar Pathøbayøløji mar Akïlïlu Lemma. A kwäänö bäät täw cwïny B, na näk bee öölö mar jööt dëël mo dwøng ya atut Gambëëla. Warkan gääp dhøgi man bee per mëë moa näk kanya lwaarge yie can nee nutge yi kwäänö no obwöre moge poot ena re dwädi ma 9-12 ni mëë moøgø cäädhö kar cööp no obwöre moge jïtge ki køny. Tier kwäänö man bee: **"Nyaay mar Täw Cwïny B Bäät Mëë mo Ci kwör Tïïë mo JööT Dëël Yi Päány Gambëëla: Muuö ki bang Mïïö ni Cøøa bang Nyilaal Ki Jammi møøk mo Gääbö**,,.

Gïna pereleth døc ni manynyi yi kwäänö man bee per ngäc lïng öölö man näk okal täw cwïny B nee rang ni ngäc jammi moo käl täw cwïny B ki moo tïïc gø nee muue ni e aay ki bang mïïö ni cøøa bang nyilaal. Manøgø kunynyö kiper joot jïëththe mo thura karge kiper män täw manøgø.

Yi Wääth mar Kwäänö: Jøa ïthge met nee nutge yi kwäänö da pïëc ki løk-pïëc mo di tïïö ki ge kiper digiige 15-20. Leere mo luup øt jaath moge thwøø di pëënynyö. Køøre, kiper ngäc nut mar täw cwïny B, remø mo thiinh (5ml) di kälø re ngatimannø. Ninäk remø mano käl nut mar täw ee nyoodhø, kunynyö kiper nee lïng muuö mar täw cwïny B ki bang mïïö ni cøøa bang nyilaal nee ngäcwa døc. Kiper manøgø nø, da rang ariew møøk mo di tïïö. Køøre, kiper nee dee gïn mo di ngääö kiper nyilaal marï, remø mo thiinh (2-3ml) dagø mo di kälø ki tier nyilaal marï ki køør met ec marï.

Køny mare ki Rääö mare: Da nwellø mo thiinh mo tägø kaateeng rääm mo thiinh, ööl dëël wala leth dëël. Bäng gwel mo di coolø ni tiir kiper nut marï yi kwäänö man. Ba, nut marï yi kwäänö man pereleth døc kiper ngäc nut mar täw cwïny B røk mëë ki dwøge piny ya atut marï. Ninäk täw cwïny B ongiiø, yï kïtha kar göök mar jööt dëël.

Nut yi Kwäänö ki køør met Ec ki Teek man en jï Dhaanhø: Luup moo coong ki bang jiy bëët bëëdö no okanø. Nyeng wala ngïï mør ba tïïc nee nëëne bäät warakatta wala yi ripøøt. Luup di coongø ki jöö mo geno gwøø ki gø ni ge bëëdö bang lwaak mar kwäänö keere. Nut marï yi kwäänö ena re met ec marï bäre. Bäng gïn mo di tïïö dëërï ki teek kiper køny mar øt-jaath mano jootï ni løny jïrï thwøø ki ma kweerï ki nut yi kwäänø man kanyo maynyï gø jaak. Ninäk da pïëc jïrï kiper kwäänø man wala yï manynya man caan gïïa joot ki køør kwäänø nee ngäyï, løny man cäänï ki ngata dwøng na kwäänö.

Nyeng: Bethelem Thecoome

Kwään Ogut: +251943504172

+251910475705

I-meel: teshebeti2016@gmail.com

Warkan Gääp Dhøgi

Yïï met ki man nudï yi kwäänö man?

- Yaa……Pööth thäängö mayya.
- Bänggø……Pwøc ngat løk pïëc oo cungngï kany.

Warakan luup man akwaana wala akwaan jïra ni lam tiere ki dhøk mana näk cøøa wïa. Ngääa ki man näk nut mara yi kwäänö man bee ki køør met ec mara ni løny man ööa wøk yie kanyo manynya jaak ni bäng gïn mo raac mo tägi dëëra. Aana jïtø ki gum dëël naa pëëa ki pïëc ni duuge jïra na karge.

Ngïï mar ngata nut yi kwäänö_____ Nïr Dwääy_____

Ngïï mar ngata dwøng re kwäänö_____ Nïr Dwääy_____

Yïïna pwøa kiper køny marï!

Warakan Met Ec/Jïëc

Yïï met niï nudï yi kwäänö man?

- Yaa…… Pööth thäängö mayya.
- Bänggø……Pwøc ngat løk pïëc oo cungngï kany.

Warakan luup man akwaana wala aana jïtø ki gum dëël oo kwaani jïra ni lam tiere thwøø ki dhøk mana näk cøøa wïa. Aana jïtø ki gum dëël mar pïëc ni jïta ki løk-pïëc mo karge. Ngääa ki man näk nut yi kwäänö man bee ki køør met ec ni løny man käla nyilaal mara wøk yi kwäänö kanyo manynya jaak. Ngääa thwøø ki man näk nut mar nyilaal mara yi kwäänö nyootha remø mo thiinh mo di kälø dëëre nee rang ni cïpe ki luup jööt dëël mo karge. Aana jïtø ki gum dëël naa pëëa ki pïëc ni duuge jïra na karge. Ngääa ki man näk luup bëët di kanø.

Ngïï mar ngata nyöödö_____ Nïr Dwääy_____

Ngïï mar ngata dwøng re kwäänö_____ Nïr Dwääy_____

Yïïna pwøa kiper køny marï!

Appendix I- Blood Sample collection and DBS preparation

Purpose- to collect blood sample under possible aseptic technique so that it can be used for the intended purpose without contamination or hemolysis.

Materials used for blood draw

- Glove
- Cotton/ Alcohol swab
- 70% Alcohol
- Tourniquet
- 5cc syringe
- EDTA

Material for DBS preparation

Glove
Lancet
Alcohol swab/cotton
Filter paper (Whatman 903)
Plastic bag
Dessicant
Drying rack

Procedure for blood draw (mothers)

After labelling the EDTA tube with the respective code of the participant by the experienced senior laboratory technologists/ principal investigator, the mother was positioned properly. After the suitable vein was identified, tourniquet was applied to make it more visible. Then the skin was cleaned using alcohol and blood was drawn. Then it was immediately transferred to the labelled EDTA tube and mixed well.

Preparation of DBS sample

After parental written consent was taken, the exposed infant was positioned properly. To warm the selected area (Big toe or finger), it was gently rubbed by their mothers/ professionals. The filter paper was labelled with unique code and date by the principal investigator. Sample was taken by an experienced trained senior laboratory technologist and principal investigator under possible aseptic technique following manual provided. After cleaning the puncture site, it was pricked using 2mm single-use lancet. The finger/ foot was held in a position so that gravity could facilitate in collection of blood in the fingertip/toe. Then the 1st large blood drop was wiped away to remove any excess body fluid. After full drop of blood was formed, the filter paper was pressed gently against the drop of blood without directly touching the paper and filled the circle completely (3-5 circle). Then cotton was used to press on the puncture site. Cessation of bleeding were ensured.

Then the DBS sample was placed on the drying rack and left overnight for air dry. Then the prepared DBS card was placed inside the gas impermeable plastic bag with 1 desiccant sachet. The bag was gently pressed to remove air before the bag was sealed. Then it was placed inside the shipping envelope with form containing the necessary information. It was stored at room temperature till transportation. When the duration of storage was greater than 2 week, it was stored at -20^{o}c (Appendix Q, Job Aid 01.00).

Appendix J- Detection of maternal HBsAg using RDT

Principle- A qualitative, solid phase, two-site sandwich immune assay for the detection of HBsAg in plasma/serum. The membrane is pre-coated with anti-HBsAg antibodies on the test line region. During testing, the plasma/serum react with the particle coated with anti-HBsAg antibodies. The mixture migrate upward on the membrane chromatographically by capillary action to react with anti-HBsAg antibodies on the membrane and generate a colored line.

Materials and equipment

- HBsAg rapid test kit (Beright diagnostic kit)
- 5cc syringe
- Glove
- 70% alcohol and cotton
- EDTA tube
- Centrifuge
- Pipette

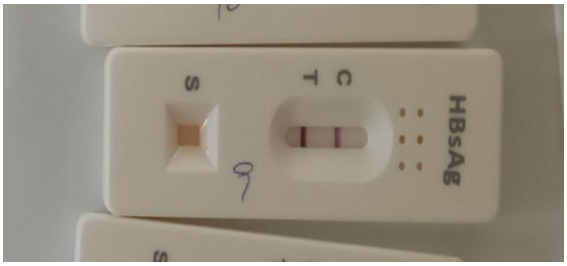
Procedure

- From selected participants, 5ml of venous blood were collected by an experienced senior laboratory technologists. The sample was immediately transferred to the labelled EDTA tube. All plasma samples were separated within 5min of sample collection by centrifugation of whole blood at 3000rpm for 5-10min. At health center where centrifuge machine were not available (Newland health center), the serum sample was separated after clot was formed naturally. Then using pipette, 3 drops of plasma sample was transferred to the sample well of the test cassette (Beright diagnostic kit, Hangzhou Alltest Biotech,

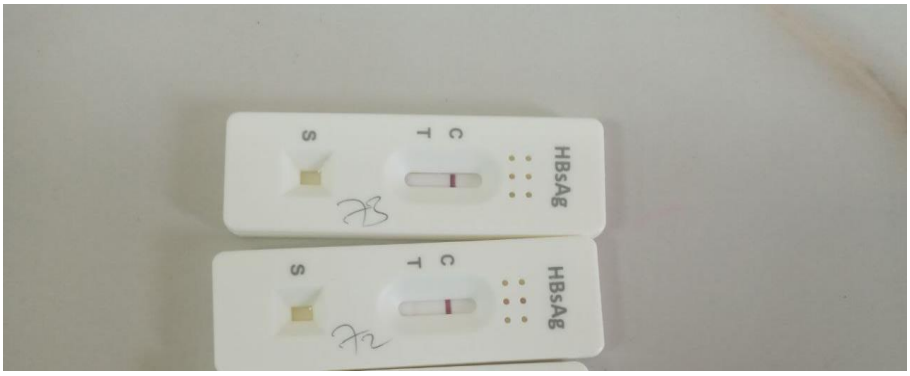
Hangzhou, P.R.China) and timer was started. All results were read between 15-30min of procedure.

Interpretation of result was as follows:

- Positive- when two distinct red colored lines appeared on both test and control region. According to the manufacturer's instructions, any shade of red color (strong or weak) on the test line was considered positive.



- Negative- when only red colored line appeared on the control line. The control line also served as procedural control indicating that proper volume of sample was added



- Invalid- if no control line appeared.

Appendix K-Detection of infant HBsAg using RDT

Principle- the membrane is pre-coated with mouse monoclonal anti-HBsAg pool on the test line region and mouse monoclonal anti-chicken IgY on the control line region. During testing, the specimen is allowed to react with the colored conjugate (mouse monoclonal anti-HBsAg conjugated gold colloid) which was pre-coated on the test strip. The mixture (mouse monoclonal anti-HBsAg +HBsAg in specimen) then moves upward on the membrane chromatographically by capillary action. For a reactive result, a purple colored line with the antibody-antigen-antibody gold particle complex will form in the test region of the result window. Regardless of the presence of HBsAg, chicken IgY conjugated gold colloid, pre- coated on the test strip, continues to move across the membrane to immobilized mouse monoclonal anti-chicken IgY, then a purple colored line in the control line region of the result window appears.

Materials and equipment

- HBsAg test kit (Bioline™ HBsAg WB kit)
- Glove
- Lancet
- Alcohol swab/ cotton
- Capillary tube/ micropipette
- Buffer (Abbott Assay Diluent)

Procedure

- The exposed infants was positioned properly and in order to warm the selected area (Big toe or finger), it was gently rubbed by their mothers/ professionals. Sample was taken by an experienced trained senior laboratory technologist and principal investigator. After cleaning the site, it was pricked using lancet. Then the 1st large drop was wiped away to avoid false result. After full drop of blood was formed, micropipette was used to transfer the sample to the test kit. 100 µl of whole blood was transferred to the specimen well marked as ‘S’. Then 3 drops of buffer (Abbott Assay Diluent) was added to carry the sample to the result window and timer was started. All results were read at 20 minutes.

Interpretation of result was as follows

- Positive/ reactive- if purple colored line appeared on both test (T) and control (C) line. Similarly, the presence of any shade of purple colored red lines was considered as stated in the manufacturer's instruction.
- Negative/ non-reactive- if only the control line appeared within the result window, then the result was reported as negative or non-reactive. It is also used as verification that sufficient volume of sample was used and proper flow had been obtained.

- Invalid- when the control line is invisible within the result window.

Appendix L -Detection of maternal HBeAg using CMIA

Principle

- The Abbott Alinity i system uses chemiluminescent microparticle immunoassay technology for qualitative detection of HBeAg in serum/plasma. It is based on two step immunoassay where paramagnetic microparticles coated with monoclonal anti-HBe antibodies bind to HBeAg present in the sample. Upon triggering a chemiluminescent reaction, the emitted light is measured in Relative Light Units (RLU). The amount light produced is proportional to the amount of HBeAg in the sample, allowing for a qualitative interpretation.

Materials and equipment

- Abbott Alinity i HBeAg reagent kit (containing microparticle coated with anti-HBe antibodies, Acridinium-labeled conjugate, sample diluent, positive& negative control)
- Calibrator
- Abbott Alinity i immunoassay analyzer
- Micropipette
- Sterile pipette tips
- Barcode scanner
- Glove

Procedure

- The positive HBsAg sample was brought to room temperature prior to procedure. Then the plasma/serum was loaded together with reagents, controls &calibrators to the Alinity i analyzer. The instrument's software was used to select the HBeAg assay. Then the analyzer automatically performed mixing of samples with coated micropartices; incubation& washing the unbound sunstances. Then adding acridium-labeled conjugate to perform second incubation. Finally trigger the chemiluminescent reaction then measure the light emission in RLU.

Interpretation

- Positive (Reactive)- HBeAg detected

- Negative (Non-reactive)- HBeAg not detected
- Invalid- requires repeat testing

Appendix M -Quantification of maternal viral load using Xpert ®HBV viral load test

Principle- An automated test for quantitative detection of the hepatitis B virus. The gene xpert instrument systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using RT-PCR. Because the cartridges are self-contained, cross contamination between samples is minimized. The xpert HBV VL test includes reagents for the detection of HBV DNA in specimens as well as two internal controls used for quantitation of HBV DNA. The internal controls are also used for adequate processing of the target &to monitor the presence of inhibitors in the PCR reactions. The probe check control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity and dye stability.

Materials and equipment

- Gene Xpert machine (Cepheid Xpert Viral Load, Sweden)
- Computer with GeneXpert Software Version 6.5
- Single use disposable cartridge (containing beads, lysis reagent(Guanidinium Thiocyanate), rinse reagent, elution reagent, binding reagent, proteinase K reagent)
- Disposable 1ml transfer pipette
- Barcode scanner
- Gloves
- Pipette

Procedure

- The positive HBsAg plasma sample stored at -20^{o}c was brought to room temperature until completely thawed and then vortexed for 10 seconds prior to use. 1ml of plasma was transferred into the labelled test cartridge using 1ml pipette. After opening the geneXpert system window, the test was initiated by clicking **Create test** then proceeded to scanning the respective cartridge barcode and entered the sample unique ID. Clicking on **start test,** the cartridge was loaded by opening the instrument module door that had blinking green light. After closing the door, the test started signaled with cessation of the blinking light.

The test status was displayed on the computer including the progress, remaining time, status and result with respect to the sample ID. When the test finished, the green light would turned off. The machine can take up to 4 test at a time. Therefore, the average time to receive a result would be 1hr if more than one test was run. The results were expressed as HBV DNA copies/ml (IU/ml) or HBV not detected. In case of Invalid and Error report repeating the test is suggested.

Appendix N -Infant HBV viral load using gene Xpert

Principle- same as maternal HBV VL testing.

According to manufacturer instruction each circle of Whatman 903 card holds 75-80µl of whole blood. Assuming the median hematocrit (0.43) of the general population, the input plasma from one spot will be 43µl (75µlx0.57). Therefore, the volume of plasma and DBS will be 1000µl and 43µl respectively. Thus the viral load on DBS should be multiplied by 23.3 (Fiseha *et al*., 2023).

Material and equipment

- Glove
- Lancet
- Alcohol swab
- Filter paper (75 µl Whatman 903, Norway)
- Plastic bag
- Desiccant
- Drying rack

Procedure

- During PCR, one whole spot of DBS sample was cut using scissor with cleaning after each cut. Then was put inside a 1.8ml cryotube and added 1000 µl of Phosphate-Buffered saline (PBS) and was vortexed for 1min to assist elution. It was left overnight at room temperature for complete elution. Then 1ml of sample was inserted to the labelled cartridge. After opening the geneXpert system window, the test was initiated by clicking **Create test** then proceeded to scanning the respective cartridge barcode and entered the sample unique ID. Clicking on **start test,** the cartridge was loaded by opening the instrument module door

that had blinking green light. After closing the door, the test started signaled with cessation of the blinking light. The test status was displayed on the computer including the progress, remaining time, status and result with respect to the sample ID. When the test finished, the green light would turned off. The machine can take up to 4 test at a time. Therefore, the average time to receive a result would be 1hr if more than one test was run. The results were expressed as HBV DNA copies/ml (IU/ml), HBV not detected, Error or Invalid.

(Grüner *et al*., 2015) (Ceesay *et al*., 2024)

Appendix O– Pictures while data and sample collection, processing

Pictures taken while data collection

Pictures taken while blood draw

Pictures captured while taking DBS sample

Pictures captured while HBsAg test and storage of HBsAg positive sample in cryotube

Appendix P- Approval letters

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አዲስ አበባ ፡ ኢትዮጵያ
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Fax: 251-11-2755296

ADDIS ABABA UNIVERSITY
Aklilu Lemma Institute of Pathobiology (ALIPB)
Addis Ababa, ETHIOPIA
251-11-276-30-91/213-57-25
e-mail: aklilu.lemma@aau.edu.et

Aklilu Lemma Institute of Pathobiology Institutional Research Ethics Review Committee (ALIPB-IRERC)

Ethical Clearance Certificate

Ref. No.: ALIPB IRERC/168/2017/25
Date: January 10, 2025

Title of the project: "Prevalence of hepatitis b virus infection, mother-to-child transmission and associated risk factors among perinatal mothers attending Gambella health facilities, south-western Ethiopia"

PI: Bethelhem Teshome,
Recommendation of the ALIPB-IRERC
Dear: Bethelhem,

The ALIPB-IRERC has reviewed your above mentioned Research Proposal and noted its merit. The IRERC would like to remind you as the PI to submit progress reports of the work every 6 months and the final report upon completion of the study. Furthermore, you are expected to notify the ALIPB-IRERC ahead of time any amendments or modifications in the protocol or premature suspension or termination of the study.

STATUS: Approved

Needs NRERB clearance: **Yes: ___No:___x___**

IRERC Chairperson: Berhanu Erko, Prof.
Signature:

IRERC Secretary: Esayas Aklilu, PhD.
Signature:

Approval
Name: **Professor Mengistu Legesse, Director**
Signature:
Date: 10/01/20

አክሊሉ ለማ የፓቶባዮሎጂ ኢንስቲትዩት Aklilu Lemma Institute of Pathobiology ADDIS ABABA UNIVERSITY

Cc// IRERC office

Support letter to Gambella Health Bureau

አክሊሉ ለማ ፓቶባዮሎጂ ተቋም
Aklilu Lemma Institute of Pathobiology

Ref ALIPB/524/17
Date 14/01/2025

ለ ጋምቤላ ክልል ጤና ቢሮ
ጋምቤላ

ጉዳዩ፡- ትብብርን ስለመጠየቅ

አክሊሉ ለማ ቆጵባዮሎጂ መኸነጥናት በሀገራቱ የተለያዩ ሆስፒታሎች ጋር በመተባበር በሂፓታይትስ ቢ በሽታ ላይ የተለያዩ ጥናቶችን በማካሄድ ላይ ይገኛል። ከዚህ ጋር በተያያዘ የመኸነጥናታችን የድህረ-ምረቃ ተማሪ የሆነችዉ ዶ/ር ቤተለሄም ተሾመ የሂፓታይትስ ቢ በሽታ ከእናት ወደ ልጅ የመተላለፍ መጠን በሚመለከት "Prevalence of Hepatitis B virus infection, mother-to-child transmission and associated risk factors among perinatal mothers attending Gambella health facilities, South Western Ethiopia" በሚል ርእስ የ MSc ጥናት ፕሮጀክት ለማካሄድ በመኸነጥናቱ ፀድቆ (Ref. ALIPB IRERC/168/2017/25) ስራዉን ለመጀመር ዶ/ር ቤተለሄም ጋምቤላ ክተማ ትገኛለች።

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ከሰላምታ ጋር

ፕ/ር መንግስቱ ለገሰ
አክሊሉ ለማ ፓቶባዮሎጂ መኸነጥናት ዲሬክተር

መንግስቱ ለማ ሌጌሴ (ፕሮፌሰር)
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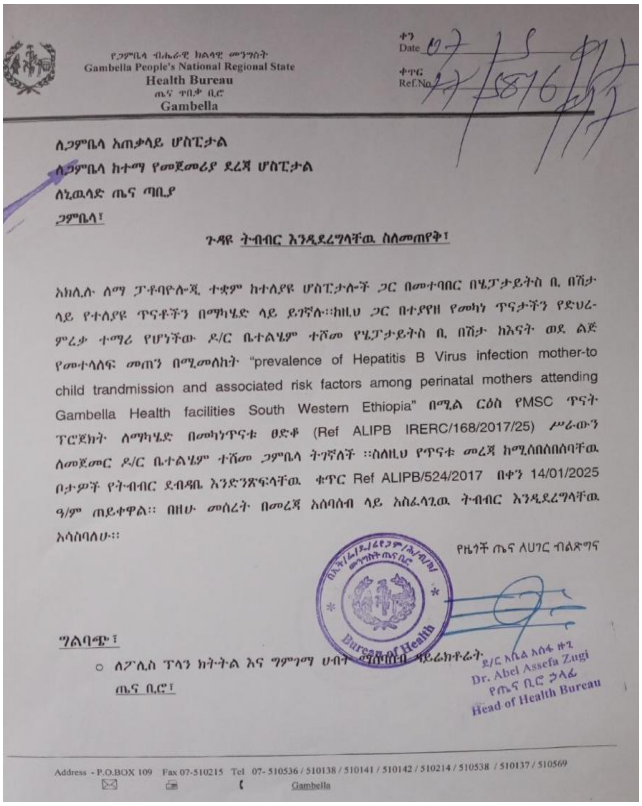
መኸነጥናት ዳይሬክተር

ኮፒ፡- ለ ዶ/ር ቤተለሄም ተሾመ

+251-11-1-2763091 aklilu.lemma@aau.edu.et aau.edu.et
SEEK WISDOM , ELEVATE YOUR INTELLECT, AND SERVE HUMANITY

Official permission and support letter to each health facility



የጋምቤላ ብሔራዊ ክልላዊ መንግስት
Gambella People's National Regional State
Health Bureau
ጤና ቢሮ
Gambella

ቀን Date 07
ቁጥር Ref.No 7/5816/17

ለጋምቤላ አጠቃላይ ሆስፒታል
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ለኒዉላድ ጤና ጣቢያ
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ጉዳዩ ትብብር እንዲደረግላቸዉ ስለመጠየቅ፣

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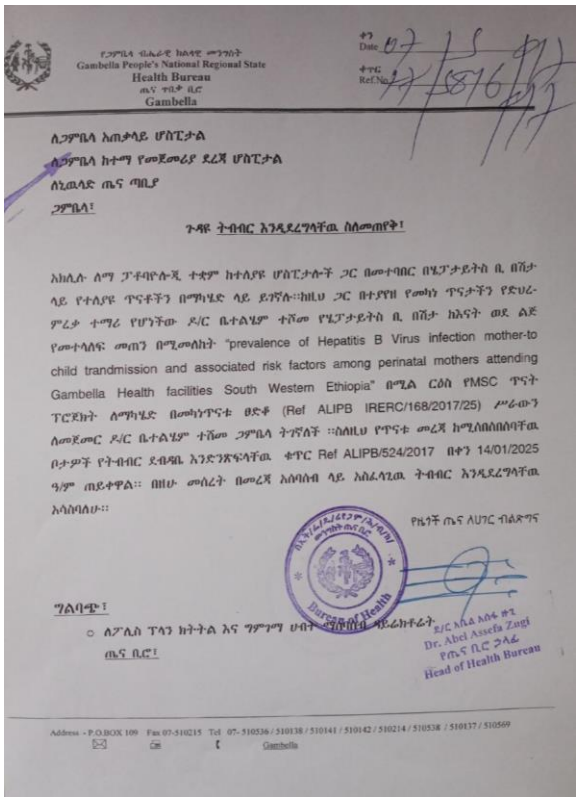

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Gambella People's National Regional State
Health Bureau
ጤና ቢሮ
Gambella

ቀን Date 07
ቁጥር Ref.No 7/5816/17

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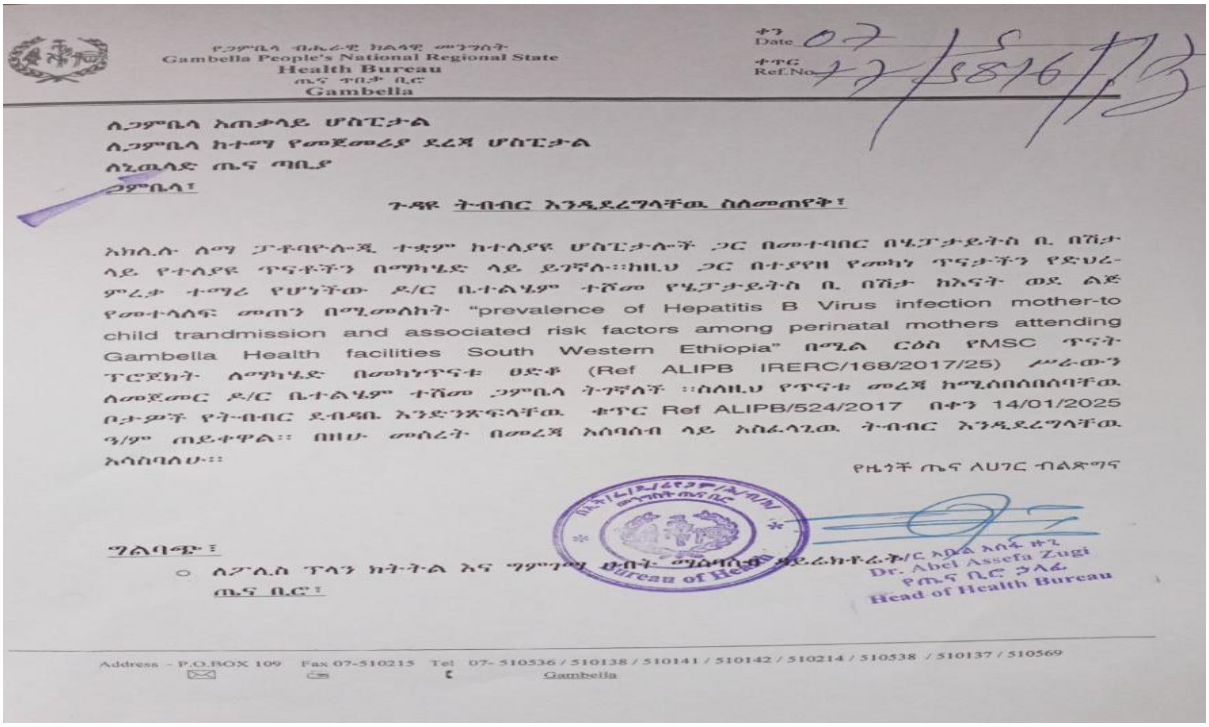

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Appendix Q–DBS preparation adopted from DBS preparation for HIV diagnosis and VL testing

Job Aid: Preparation and Packaging of Dried Blood Spots (DBS) for Pediatric (4 weeks-10 yrs.) HIV Diagnosis or Viral Load Testing

(Last Revised: 4 May, 2020; guidance sourced from US CDC, CLSI and WHO; reviewed by USAID, US CDC and PEPFAR)

1. Collect required supplies

- GHSC DBS Kit for pediatric HIV diagnosis or viral load testing
- Pen
- Clinic register
- Shipping envelopes
- Sharps container
- Lab requisition form
- Paper towels/bench protectors

Warnings and precautions

- **Only powder-free gloves must be used**
- **Gloves must be changed, and hands must be washed and dried between each client and before touching or labelling the DBS card**
- **A clean paper towel/bench protector must be used between each client for placing DBS kit Items.**
- **On neonates (1-30 day old), it is safer to use a 0.85 x 1.75 mm single use safety lancet**
- **Dispose all sharps and biohazardous waste in designated disposal bins immediately following use.**

2. Complete all necessary paperwork for the patient

- Clinic registers
- Laboratory request form
- DBS card
- Labels

3. Choose the area to be pricked and ask the helper to warm this area by gentle rubbing with hands.

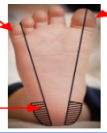
Recommendation for area to be pricked:

- Infants 4 weeks – 4 months: **heel**
- Infants 4 months – 10 months: **big toe**
- Infants > 10 months or > 10 kg - 10 yrs: **finger**

The puncture should be made on either side of the finger tip.

Arrows point to fingers and areas marked as safe to prick

Hatched areas are safe to prick



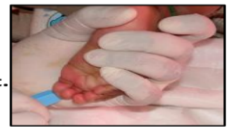
4. Clean the spot to be pricked with an alcohol wipe and allow to dry for 30 seconds.

5. If drawing blood from the big toe or finger, position the infant with the foot or hand pointing down.

6. Gently squeeze the area to be pricked.

7. Prick the infant in the selected spot with a single use 2 mm lancet.

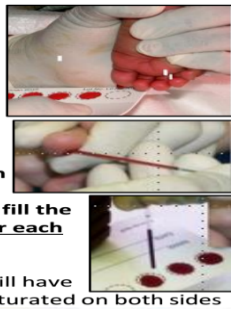
8. Wipe away the first blood drop and allow a large blood drop to collect.



9. Options for transferring blood to the DBS Card

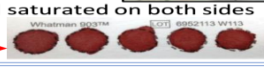
9a. Touch the filter paper gently against the drop of blood and completely fill the circle. OR

9b. Hold the capillary tube horizontal to the site being bled and touch the tube tip to the blood drop. Fill the tube to the specified volume and gently touch the blood at the edge of the tube against the paper and completely fill the circle. Use a new capillary tube for each blood transfer.



10. Check for validity of the DBS specimen: a valid DBS specimen will have 3-5 circles completely filled and saturated on both sides of the card .

A valid DBS specimen



11. Place DBS cards on a drying rack – use a separate rack for HIV Diagnosis and for Viral Load

- Do not let the card touch any other object
- Let air dry at room temperature for at least 4 hours or overnight up to 24 hrs.
- Avoid exposing the card to direct sunlight.
- Keep lab request forms with DBS cards.

12. Clean the puncture site and press cotton wool against it until bleeding stops. Ensure the bleeding has stopped for at least 5 minutes.

13. Packaging dried DBS cards

- Place each DBS card first in a glassine envelope and then in a gas-impermeable bag
- Place 3 desiccant sachets and 1 humidity indicator card in each bag
- Gently press the bag to remove air before sealing the bag.
- Place the bag, lab forms and specimen delivery checklist into the shipping envelope.

Note: DBS cards prepared this way are stable up to 2 weeks at room temperature. If they cannot be shipped within 2 weeks, place them at -20°C or -70°C freezer.