Journal of Clinical Virology 179 (2025) 105826

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Prevalence of hepatitis B virus infection among pregnant women and cord blood hepatitis B surface antigen positive newborns in sub-Saharan Africa and South Asia

Carine Bokop [a], Nisha Dhar [a,b], Alane Izu [a], Jayendrie Thaver-Kleitman [c], Nishi Prabdial-sing [c], Musa Mohammed Ali [d], Godwin Akaba [e], Hellen C. Barsosio [f,g], James A. Berkley [g,h], Manisha Madhai Beck [i], Tolossa E. Chaka [j], Clare L. Cutland [k], Phurb Dorji [l], Maksuda Islam [m,n], Adama Mamby Keita [o], Feleke Belachew Lema [p], Nubwa Medugu [q,r], Stella Mwakio [g], Stephen Obaro [q,s], Eyinade K. Olateju [t], Rani Diana Sahni [u], Samir K. Saha [m,n], Sridhar Santhanam [v], Ragunath Sharma [l], Betuel Sigaúque [w], Eric A.F. Simoes [x], Samba O. Sow [o], Milagritos D. Tapia [y], Balaji Veeraraghavan [u], Gaurav Kwatra [a,b,u,z,*,1,2], Shabir A. Madhi [a,k,aa,2]

[a] South Africa Medical Research Council Vaccines and Infectious Diseases Analytics Research unit, Faculty of Health Science, University of the Witwatersrand, Johannesburg, South Africa
[b] Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
[c] National Institute for Communicable Disease, Centre for Vaccine and Immunology, Johannesburg, South Africa
[d] Hawassa University College of Medicine and Health Sciences, School of Medical Laboratory Science, Hawassa, Ethiopia
[e] Department of Obstetrics and Gynaecology, The University of Abuja Teaching Hospital, Abuja, Nigeria
[f] Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK
[g] KEMRI/Welcome Trust Research Program, Kilifi, Kenya
[h] Centre for Tropical Medicine & Global Health, Nuffield Department of Medicine, University of Oxford, UK
[i] Fetal Medicine Unit, Department of Obstetrics and Gynaecology, Christian Medical College, Vellore, India
[j] Department of Pediatrics and Child Health, Adama Hospital Medical College, Adama City, Ethiopia
[k] African Leadership in Vaccinology Expertise, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
[l] Jigme Dorji Wangchuck National Referral Hospital, Thimphu, Bhutan
[m] Child Health Research Foundation, Dhaka, Bangladesh
[n] Bangladesh Shishu Hospital and Institute, Dhaka, Bangladesh
[o] Le Centre pour le Développement des Vaccins du Mali (CVD-Mali), Bamako, Mali
[p] Oromia Pubic Health Research, Capacity Building and Quality Assurance Laboratory, Adama City, Ethiopia
[q] International Foundation against Infectious Diseases in Nigeria, Abuja, Nigeria
[r] Department of Medical Microbiology and Immunology, Nile University of Nigeria, Abuja, Nigeria
[s] Division of Pediatric Infectious Diseases University of Alabama at Birmingham, USA
[t] Department of Paediatrics, University of Abuja Teaching Hospital, Abuja, Nigeria
[u] Department of Clinical Microbiology, Christian Medical College, Vellore, India
[v] Department of Neonatology, Christian Medical College, Vellore, India
[w] Centro de Investigação em Saúde da Manhiça (CISM), Manhica, Mozambique
[x] Department of Pediatrics, Children's Hospital Colorado, University of Colorado School of Medicine, Colorado School of Public Health, Aurora, CO, USA
[y] Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, USA
[z] Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA
[aa] Wits Infectious Diseases and Oncology Research Institute, Faculty of Health Science, University of the Witwatersrand Johannesburg, South Africa

* Correspondence to: South Africa Medical Research Council Vaccines and Infectious Diseases Analytics Research Unit, Chris Hani Baragwanath Academic Hospital, Wits Learning Centre, Chris Hani Road, 11th Floor West Wing, 2013 Bertsham, South Africa.
E-mail addresses: gaurav.kwatra@wits-vida.org, Gaurav.Kwatra@cchmc.org (G. Kwatra).
[1] Present address: Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229, USA.
[2] Equal contribution.

https://doi.org/10.1016/j.jcv.2025.105826
Received 6 March 2025; Received in revised form 4 June 2025; Accepted 10 June 2025
Available online 11 June 2025

ARTICLE INFO

Keywords:
Seroprevalence
Hepatitis B virus
Pregnant women
Newborns

ABSTRACT

Background: Newborns infected with Hepatitis B Virus (HBV) are at risk of chronic liver disease and hepatocellular carcinoma.
Objectives: This study investigated the prevalence of HBV infection among pregnant women and cord blood Hepatitis B surface antigen (HBsAg) positivity of their newborns in Bangladesh, Bhutan, India, Ethiopia, Mozambique, Kenya, Nigeria, Mali, and South Africa.
Study design: Randomly selected paired maternal and cord blood samples (n = 101 each site) taken at delivery were tested for HBsAg and Hepatitis B extractable antigen (HBeAg) in the women using a chemiluminescent microparticle immunoassay. Similarly, cord blood sample of newborn was assessed for HBsAg reactivity. HBV DNA was quantified using the Xpert® HBV viral load assay, followed by genotyping.
Results: The overall prevalence of maternal HBsAg positivity was 5.5 % (95 %CI: 0.4 %–7.1 %; n = 50/909). HBsAg positivity was higher in African countries (7.3 %; 95 %CI: 5.4 %–9.6 %; n = 44/606) compared to South Asian countries (2.0 %; 95 %CI: 0.8 %–4.3 %; n = 6/303; p = 0.002). Relative to South Africa, there were higher odds of HBsAg sero-positivity in women from Mozambique ((aOR): 7.7, 95 %CI: 1.6 %–37.8 %) and Mali (aOR: 5.7; 95 %CI: 1.1 %–29.7 %). The rate of HBsAg positivity in cord blood of babies born to HBsAg positive women was 28.0 % (95 %CI: 17.1 %–42.3 %; n = 14/50), including 31.8 % (95 %CI: 19.5–47.4 %; n = 14/44) in African countries. No cord blood HBsAg positivity was observed in South Asia. Genotypic analysis revealed HBV genotypes A (41.7 %) and E (58.3 %) were pre-dominant.
Conclusion: The high rate of cord blood positivity (28.0 %) for HBsAg underscores the urgency of enhancing HBV prevention strategies to meet the World Health Organization's target of a 90 % reduction in new HBV infections by 2030.

1. Introduction

Infection with hepatitis B virus (HBV) may cause acute illness, as well as chronic infection, as evidenced by the persistence of HBV surface antigen (HBsAg) beyond six months after infection [1]. The risk of chronic HBV infection declines with ascending age of infection, being 90 %, 20–60 %, and less than 5 % following infection in the newborn period, at five years of age, and during adulthood, respectively [1–3]. In 2022, there was an estimated 254 million individuals with chronic HBV liver disease, accounting for over 1.1 million deaths annually [4]. The World Health Organization (WHO) has set a target to reduce new hepatitis infections by 90 % between 2016 and 2030 [5]. Nevertheless, HBV infection remains a major public health challenge, particularly in low and middle-income countries (LMIC) [6]. Seventy percent of HBV infections occur in African children under 5 years of age, with 4.5 million children estimated to be living with HBV infection in 2020 [7].

Mother-to-child transmission of HBV may occur *in utero*, intrapartum, or during the post-natal period [8]. The presence of HBsAg, Hepatitis B extractable antigen (HBeAg), or HBV DNA in the cord blood of newborns is indicative of intrauterine exposure to HBV [1,9]. Mother-to-newborn transmission of HBV accounts for over 50 % of individuals with chronic HBV infection in HBV-endemic settings [10,11]. While, several low- and middle-income countries have adopted policies supporting HBV screening during antenatal care, routine and consistent implementation of such policies remains limited in many settings due to resource and programmatic constraints [4,12]. Furthermore, Hepatitis B immunoglobulin G (HBIG) is not readily accessible in LMIC, and as of 2019–2021, 73 % (35/48) of sub-Saharan African and 27.3 % (3/11) of Southeast Asian countries had not implemented a birth dose of HBV vaccine [13–15]. This study aimed to evaluate the prevalence of HBV infection in women at the time of delivery and the rate of cord blood HBV positivity in newborns across six African and three South Asian countries.

2. Materials and methods

2.1. Study design and participants

This study was conducted in Bangladesh, Bhutan, Ethiopia, India, Kenya, Mali, Mozambique, Nigeria, and South Africa. The women had been recruited from January 10, 2016 to Dec 11, 2018 during the early stages of labour; and mother-newborn dyad blood samples had been collected as part of a larger study undertaken to evaluate colonization with Group B streptococcus (GBS) and GBS sero-epidemiology [16]. In the parent study [16], a multicountry, prospective, observational study was conducted across 11 maternity and obstetric care facilities. Participant recruitment was facility-based, enrolled at public health facilities. All eligible pregnant women who presented at the facilities during the daytime, Monday to Friday, were approached for participation in the study, and were recruited from an urban (e.g., Mali and South Africa), rural (e.g., Bangladesh, Kenya, Mozambique, and Bhutan), and mixed urban-rural areas (e.g., India, Ethiopia, and Nigeria). Eligible participants were pregnant women aged 18–45 years, enrolled at ≥37 weeks of gestation, and confirmed to be HIV negative. Exclusion criteria included the presence of underlying medical conditions, receipt of intrapartum antibiotics, or blood transfusion within 30 days prior to delivery. At each site, 101 participants were enrolled in the parent study [16]. Participants' HIV status was obtained from their medical records if tested during the current pregnancy; if it had not, they were tested through routine care services before enrollment in the study. Demographic data of the mothers and infant-related factors, including gender and birth weight were collected. Although, specific data on HBV prevention practices (e.g., antiviral prophylaxis) were not collected, data on Hepatitis B vaccination strategies and coverage in participating countries were collected at the time of study sample collection. In the current study, all the participants who had consented to the storage and further use of their blood and cord blood samples were included. Venous blood samples were collected from the women by trained nurses at the time of delivery and cord blood samples were obtained immediately after delivery. Samples collected were immediately centrifuged at the site to extract serum. The aliquoted serum samples were archived at Wits-VIDA at a temperature of −70 °C.

2.2. Sample size

The sample size was calculated based on the reported prevalence of HBsAg among pregnant women in Africa ranging from 1.9 % to 16.2 % in 2017 [17]. Assuming at least 7 % prevalence of HBsAg among pregnant women, we targeted to test 101 pregnant women at each study site to detect at least seven HBsAg positive cases with ±5 % precision per site. An epidemiological tool calculator (Epitools) was used to estimate the final sample size.

2.3. Laboratory procedure

Detection of HBsAg in the serum of women and cord blood of newborns was performed using the ARCHITECT *i*1000SR System reagent kit (Abbott ARCHITECT® HBsAg, Sligo, Ireland) as per the manufacturer's guideline. Paired cord blood testing was restricted to women tested positive for HBsAg. Results were expressed as the sample's relative light units (RLUs) to the cut off RLU i.e. Signal to cut off (S/CO), with non-reactive defined as S/CO <1.00 and reactive as S/CO ≥1.00. For all the women positive for HBsAg S/CO >1 (50 cases), both HBV DNA and HBeAg were quantified. The detection of HBeAg in serum samples of pregnant women followed a similar protocol using the system reagent kit (Abbott ARCHITECT® HBeAg, Sligo, Ireland) as per the manufacturer's guidelines. The HBV DNA concentration in women testing HBsAg positive was quantified using the GeneXpert® HBV Viral Load (Cepheid AB, SE-171 54 Solna, Sweden). Automated interpretation utilized fluorescent signals and embedded calculation algorithms, reporting HBV DNA detected or undetected, with a detection range of 10 (i.e. lower limit of detection) to 1 × 109 international unit per millilitre (IU/ml). Sequencing was restricted to samples with an HBV viral load ≥200 IU/ml. Viral DNA was extracted from 200 μL of serum using the QIAamp DNA Mini Blood Kit (Qiagen, Germany). High maternal HBV DNA copies has been associated with greater risk of HBV *in utero* transmission, with maternal HBV DNA >6 $\log^{10}$ copies/ml (≥200,000 IU/ml associated with increased risk of *in utero* HBV infection [1,18], hence ≥200,000 IU/ml was considered as one of the factors in our analysis. Nucleotide BLAST analysis was conducted on the HBV sequences to identify homologous sequences, and the phylogenetic tree was inferred using the Neighbor-Joining method [19]. To detect mutations, reference sequences used to assess the mutations was the HBV genome sequence with accession number NC_003977.2 [20]. Detailed information on the sequencing methods, including primers and probes are provided in Supplementary material.

2.4. Statistical analysis

Descriptive analysis was reported as proportion and categorical variables were compared using Chi-square analysis. Sero-positivity rates were calculated as proportions with 95 % confidence intervals, estimated using the exact (Clopper-Pearson) method for binomial distributions. Logistic regression analysis was performed to explore predictors of HBsAg positivity among women. Crude odds ratio (cOR) and adjusted odds ratio (aOR) with 95 % CIs were calculated using univariate and multivariable logistic regression analysis in Stata. All statistical analyses were performed on STATA version 18 (StataCorp LP, Texas, USA). A p-value of ≤0.05 was considered statistically significant. The study was approved by the Human Research Ethics Committee (HREC) at the University of the Witwatersrand in Johannesburg, South Africa (HREC M210850).

3. Results

A total of 909 mother-newborn dyad samples were analysed, including 606 from African countries and 303 from South Asian countries. The overall mean age of women was 25.8 (SD ± 5.2) years, Table 1.

Overall, 5.5 % (95 %CI: 0.4 %–7.1 %; n = 50/909) of the women were sero-positive for HBsAg, with overall prevalence being higher in women from Africa (7.3 %; 95 %CI: 5.4 %–9.6 %; n = 44/606) compared with South Asian countries (2.0 %; 95 %CI: 0.8 %–4.3 %, 6/303; p = 0.004); Table 2. The prevalence of HBsAg sero-positivity in the women varied across the African countries, being the highest in Mozambique

Table 1
Demographics and baseline characteristics of pregnant women (n = 101 at each site) analysed in this study.

	Bangladesh n (%)	Bhutan n (%)	Ethiopia n (%)	India n (%)	Kenya n (%)	Mali n (%)	Mozambique n (%)	Nigeria n (%)	South Africa n (%)	Total n (%)
Age group										
18–25	67 (66.3)	43 (42.6)	62 (61.4)	52 (51.5)	75 (74.3)	59 (58.4)	67 (66.3)	16 (15.8)	58 (57.4)	499 (54.9)
26–35	31 (30.7)	54 (53.5)	34 (33.7)	49 (48.5)	24 (23.8)	36 (35.6)	29 (28.7)	70 (69.3)	38 (37.6)	362 (40.1)
36–45	3 (2.9)	4 (3.9)	5 (4.9)	0 (0.0)	2 (2.9)	6 (5.9)	5 (4.95)	15 (14.9)	5 (4.9)	45 (4.9)
Maternal age, mean (SD), y	23.7 (±4.9)	26.9 (±4.5)	25.4 (±4.7)	25.6 (±3.8)	24.9 (±3.7)	25.5 (±5.4)	24.6 (±5.7)	30.7 (±4.7)	25.2 (±5.6)	25.8 (±5.2)
Parity										
Primigravida	0 (0.0)	0 (0.0)	17 (25)	0 (0.0)	17 (19.5)	0 (0.0)	10 (12.35)	0 (0.0)	19 (25.7)	63 (10.3)
1–2	49 (89.9)	33 (76.7)	42 (61.8)	45 (88.2)	48 (55.2)	30 (40.5)	41 (50.62)	44 (55.0)	46 (62.2)	378 (61.7)
≥3	6 (10.9)	10 (23.3)	9 (13.2)	6 (11.8)	22 (25.3)	44 (59.5)	30 (37.04)	36 (45.0)	9 (12.2)	172 (28.1)
Missing observations	46	58	33	50	14	27	20	21	27	296
Haemoglobin (g/dl)										
Normal (12- (12–16)	5 (4.9)	74 (73.3)	77 (76.2)	43 (42.6)	32 (31.7)	64 (63.4)	19 (18.81)	38 (37.6)	62 (61.4)	414 (45.5)
Mild anaemia (10.6–11.9)	21 (20.8)	25 (24.8)	19 (18.8)	43 (42.6)	17 (16.9)	27 (26.7)	35 (34.65)	36 (35.6)	26 (25.7)	249 (27.9)
Anaemic (≤10.5)	75 (74.3)	2 (1.9)	5 (4.95)	15 (14.9)	52 (51.5)	10 (9.9)	47 (46.53)	27 (26.7)	13 (12.9)	246 (27.1)
Maternal MUAC[a] (centimetre)										
Underweight (≤22.9)	8 (7.92)	6 (6.1)	19 (18.8)	6 (8.4)	14 (13.9)	6 (14.6)	5 (4.95)	0 (0.0)	4 (4.3)	68 (8.4)
Normal weight (≥23 to < 33)	90 (89.1)	90 (90.9)	14 (13.9)	57 (80.3)	79 (78.2)	30 (73.2)	88 (87.13)	90 (89.1)	73 (79.3)	611 (75.6)
Overweight (≥33)	3 (2.9)	3 (3.0)	68 (67.3)	8 (11.3)	8 (7.9)	5 (12.2)	8 (7.92)	11 (10.9)	15 (16.3)	129 (15.9)
Maternal BMI[b]										
Underweight (<18.5)	1 (0.9)	1 (0.9)	2 (1.9)	8 (7.9)	2 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)	4 (3.9)	18 (1.9)
Normal weight (18.6–24.9)	37 (36.6)	14 (13.9)	52 (51.5)	40 (39.6)	50 (49.5)	37 (36.6)	33 (32.7)	16 (15.8)	26 (25.7)	305 (33.6)
Overweight (25–29.9)	50 (49.5)	57 (56.4)	45 (44.5)	37 (36.6)	31 (30.7)	42 (41.6)	53 (52.5)	47 (46.5)	28 (27.7)	390 (42.9)
Obese (≥ 30)	13 (12.9)	29 (28.7)	2 (1.9)	16 (15.8)	18 (17.8)	22 (21.8)	15 (14.8)	38 (37.6)	43 (42.6)	196 (21.6)
Highest level of education										
No schooling	5 (4.9)	20 (20.2)	10 (10)	0 (0.0)	11 (10.9)	59 (58.4)	7 (6.9)	3 (2.9)	0 (0.0)	115 (12.7)
Primary school	19 (18.8)	9 (9.09)	51 (51)	11 (10.9)	60 (59.4)	23 (22.8)	40 (39.6)	11 (10.9)	7 (6.9)	231 (25.5)
High school	62 (61.4)	46 (46.5)	21 (21)	17 (16.8)	25 (24.7)	16 (15.8)	53 (52.5)	36 (35.6)	76 (75.3)	352 (38.8)
Tertiary	15 (14.8)	24 (24.2)	18 (18)	73 (72.3)	5 (4.9)	3 (2.9)	1 (0.9)	51 (50.5)	18 (17.8)	208 (22.9)

[a] MUAC, mid-upper arm circumference,
[b] BMI, Body mass index.

Table 2
Hepatitis B surface antigen sero-positivity among pregnant women and their newborns in Africa and South Asia.

Countries	No. of cases	HBsAg sero-positivity in mothers	Rate of cord blood HBsAg positivity	
	n/N	% (95 %CI)	n/N	% (95 %CI)
Sites				
Bangladesh	2/101	2.0 (0.4 %–7.6 %)	0/2	Not applicable
Bhutan	2/101	2.0 (0.4 %–7.6 %)	0/2	Not applicable
Ethiopia	4/101	4.0 (1.4 %–10.1 %)	1/4	25.0 % (2.2–82.3 %)
India	2/101	2.0 (0.4 %–7.6 %)	0/2	Not applicable
Kenya	5/101	5.0 (2.0–11.4 %)	0/5	Not applicable
Mali	12/101	11.9 (6.8 %–19.8 %)	7/12	58.3 % (29.0–82.7 %)
Mozambique	13/101	12.9 (7.6–20.9 %)	0/13	Not applicable
Nigeria	8/101	8.0 (4.0–15.0 %)	6/8	75.0 % (34.2–94.5 %)
South Africa	2/101	2.0 (0.4–7.6 %)	0/2	Not applicable
Overall	50/909	5.5 (0.4 %–7.1 %)	14/50	28.0 % (17.1 %–42.3 %)
South Asian countries	6/303	2.0 (0.8–4.3 %)	0/6	Not applicable
African countries	44/606	7.3 (5.4 %–9.6 %)	14/44	31.8 % (19.5 %–47.3 %)

HBsAg, Hepatitis B surface antigen; 95 %CI, 95 % confidence interval.

(12.9 %; 95 %CI: 7.6–20.9 %; n = 13/101) and Mali (11.9 %, 95 %CI: 6.8 %–19.8 %; n = 12/101), and the lowest in South Africa (2.0 %; 95 % CI: 0.4 %–7.6 %; n = 2/101); Table 2. Fig. 1 represents a geographical map illustrating the variation in HBsAg sero-positivity across the study sites, highlighting regional differences observed in the study population.

Relative to South Africa, in multivariate logistic regression analysis, there was a higher odd of being HBsAg sero-positive in women from Mozambique (aOR: 7.7, 95 %CI: 1.6 %–37.8 %, p = 0.01) and Mali (aOR: 5.7, 95 %CI: 1.1 %–29.7 %, p = 0.03) (Supplementary Table 1). In overall analysis of all the sites, there was a higher odd of being HBsAg sero-positive in women with only a primary level education compared with college level education (cOR: 2.6, 95 %CI: 1.1 %–6.3 %); Supplementary Table 1.

In women who were HBsAg sero-positive, HBV DNA was detectable in 72.0 % (36/50) of the cases; that included 100 % (12/12) of women from Mali, 100 % (2/2) from Bangladesh, 100 % (4/4) from Ethiopia, 87.5 % (7/8) from Nigeria, 61.5 % (8/13) from Mozambique, 50 % (1/2) from Bhutan, 40 % (2/5) from Kenya, and none from India and South Africa (Supplementary Table 2). Furthermore, HBeAg positivity was 14.0 % (7/50) among the women who were HBsAg sero-positive, Supplementary Table 2. Nineteen percent (7/36) of the women with detectable HBV DNA were HBeAg sero-positive. The HBeAg sero-positivity was 28.6 % (95 %CI: 4.91–75.60 %; n = 2/7) in women with HBV viral load ≥200,000 IU/ml, and HBeAg sero-positivity was 71.4 % (95 %CI: 24.39 %–95.08 %; n = 5/7) in women with HBV viral load less than 200,000 IU/ml; Supplementary Table 3. In our cohort, only 50/909 (5.5 %) of the women were born since when HBV vaccine was introduced in the routine public immunization program of their countries. Consequently, the majority of participating women (862/909; 94.8 %) were likely to have been naive for HBV vaccination (Supplementary Table 4).

The overall rate of cord blood HBsAg positivity was 28.0 % (95 %CI: 17.1 %–42.3 %; n = 14/50) in newborns of women who were HBsAg sero-positive; including 31.8 % (95 %CI: 19.5 %–47.3 %; n = 14/44) in Africa and none in South Asia (Table 2). Notably, there were no cases of cord blood HBsAg positivity in newborns of women who tested HBsAg sero-positive in Mozambique (0/13), compared with 75.0 % (95 %CI: 34.2 %–94.5 %; n = 6/8) and 58.3 % (95 %CI: 29.0 %–82.7 %; 7/12) HBV positivity in Nigerian and Malian newborns, respectively (Table 2).

Of the 14 newborns HBsAg positive in cord blood, 92.9 % (n = 13/14) of their mothers had a reactive HBV DNA, including four women who tested positive on both HBV DNA and HBeAg assays (Supplementary Table 5). Ninety-two percent (12/13) of HBsAg sero-positive newborns were born to women with reactive HBV DNA and viral load of ≤200,000 IU/ml; Supplementary Table 5. The single case of HBsAg positive newborn in whom the mother was HBsAg sero-positive, but negative for HBV DNA and HBeAg, had a high titer of HBsAg (4754.27 S/CO); Supplementary Table 5. Among the 13 Mozambican

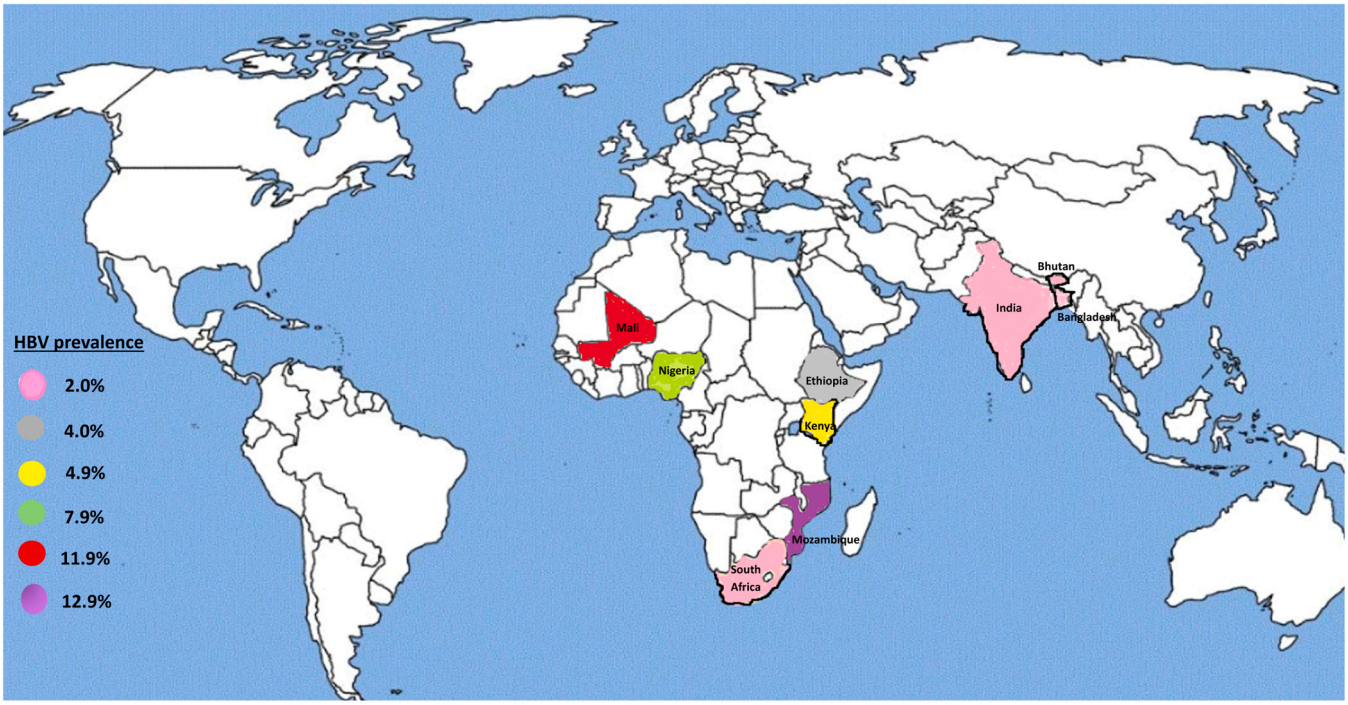

Fig. 1. Prevalence of Hepatitis B virus among pregnant women across the study sites.

women who were HBsAg sero-positive, although two women were HBeAg sero-positive and had detectable HBV viral load (≤200,000 IU/ml), there were no cases of HBsAg positivity in cord blood; Supplementary Table 5. Among the mothers with detectable HBV DNA, cord blood HBsAg positivity was observed in 33.3 % (3/9) of mothers with an HBV viral load below the lower limit of detection (<10 IU/ml); Supplementary Table 5.

3.1. Genotypic analysis results

Genotyping attempted on maternal samples with HBV DNA titers of ≥200 IU/ml; (i.e. 19 of 36), was successful in 63.2 % (12/19) of samples tested, of which 41.7 % (5/12) and 58.3 % (7/12) were genotype A and E, respectively; Supplementary Figs. 1 and 2. Among the 14 newborns who were HBsAg positive, maternal samples were successfully genotyped in only 4 cases, all of which were identified as genotype E; Table 3.

Genotype-specific similarities and mutations observed within the reverse transcriptase (RT) domain and small hepatitis B surface protein (SHB i.e. encoded by the S region of the HBV surface gene, excluding the pre-S1 and pre-S2 regions) across different countries, showed that genotype A exhibited noteworthy similarities within the RT domain, including the presence of mutations M129L, V163I, and I253V, despite being from Kenya, Bhutan, and Mozambique (Supplementary Table 6). Moreover, samples MO32, MO91, and MO69 from Mozambique shared common mutations N122H and N131D within the RT domain, indicating regional genetic similarities (Supplementary Table 6). All samples with genotype A harbored the S207N mutation in the SHB across different geographical regions. There were no gene markers associated with antiviral resistance for commonly used antiviral against HBV; Supplementary Table 6.

4. Discussion

The findings from our study corroborate previous studies on HBsAg positivity in pregnant women from other sub-Saharan African countries, including Gambia (9.2 %), Ghana (7.7 %), Ethiopia (4.4 %), and Nigeria (6.9 %) [17,21,22]. We, however, observed heterogeneity in maternal HBsAg positivity across the African region included in our study, which may be influenced by socio-economic factors, cultural practices, and the extent of HBV awareness [23]. In contrast, the lower prevalence of HBsAg positivity observed in the Southeast Asian countries is consistent with earlier studies from the region, where prevalence has been reported to range from 0.4 % in Bangladesh to 5.4 % in Bhutan [24–26]. Differences in HBV sero-positivity rates across countries may highlight the combined influence of vaccination coverage and healthcare infrastructure. Countries with high vaccination coverage at the time of sample collection, including Bangladesh, Bhutan, India, and South Africa (≥84 %) [27], consistently demonstrate low HBsAg sero-positivity rates (~2 %). However, in Kenya and Ethiopia, with moderate vaccination coverage (81 % and 68 %, respectively) [27], slightly high sero-positivity rates (5–4 %) were observed. Another informative observation was the higher HBsAg positivity rate (12.9 %) observed in Mozambique, despite the elevated vaccine coverage (85 %) [27]. The findings highlight the critical need to expand vaccine access and coverage. In addition, factors including delays in birth dose vaccination, lack of maternal HBV screening and follow-up care for infants born to HBV-positive mothers may as well contribute to this disparity. There was a counterintuitive pattern of lower HBeAg sero-positivity among women with HBV viral load ≥200,000 IU/ml as compared to those with <200,000 IU/ml, which is likely attributable to the very small number of cases in each category and the resulting imprecision, as indicated by the wide and overlapping 95 % confidence intervals. The observed distribution should therefore be interpreted with caution, and further studies with larger sample sizes are needed to clarify this relationship.

The World Health Organization (WHO) recommends screening of pregnant women for HBsAg in regions where the sero-positivity is more than 2 % [28]. In high HBV prevalence regions with limited access to HBV DNA quantification, universal TDF prophylaxis for HBsAg positive pregnant women has been recommended as a pragmatic and cost-effective alternative to a test-and-treat approach based on HBV DNA levels [4]. Our findings underscore the need for country-specific data to determine whether HBsAg screening should be routinely implemented at a country level, which would need to be coupled with interventions such as treatment of infected women with antiviral prophylaxis, as well as HBV immunoglobulin and birth doses of HBV vaccine for newborns to women who test HBsAg positive.

We observed a high rate of HBsAg positivity (31.8 %) in cord blood in Africa, which is consistent with previous studies from Tanzania (33 %) and Nigeria (24 %) [29,30]. It is important to note that the presence of HBV markers in cord blood indicates exposure to, but not necessarily infection with HBV [31]. All fetuses born to women who were HBsAg positive or had detectable HBV DNA are considered exposed. Among HBV exposed fetuses, those who were positive for HBsAg or HBV DNA in cord blood represent a sub-category of the exposed who might have transient positivity for those markers. Infants who test positive for HBV in cord blood often show a low rate of persistent infection at follow-up. Liu et al. found that while 42–49 % of neonates born to HBeAg-positive mothers had detectable HBsAg or HBV DNA in cord blood, only 3.7 % were infected at follow-up [31]. Another study observed that while 31 % of cord blood samples from HBsAg positive mothers were HBsAg positive, only 6.6 % remained positive at 6 months follow-up [32]. In our study settings, comprehensive HBV standard of care was not uniformly implemented during the period of samples collection. South Africa, Bhutan, Bangladesh, Mozambique, and Kenya had not implemented antenatal routine HBV screenings unless there were a risk factors. However, Mali, Ethiopia, India, and Nigeria have integrated routine antenatal HBV screenings as part of the national guidelines, although coverage remains low. There were no cases of cord blood HBsAg positivity in Mozambican newborns, despite the highest maternal HBsAg positivity prevalence being identified in Mozambique.

Table 3
Quantification and genotypic analysis of Hepatitis B virus among Hepatitis B surface antigen positive women.

HBsAg positive women[a] (Country and Sample ID)	HBV DNA[b] detected (IU/ml)	HBeAg[c] detected (S/CO)	HBsAg in cord blood of newborns born to genotyped HBsAg sero-positive women (S/CO)	Genotypes results of HBV infected women
Bhutan				
Bh15	1680	NR[d]	NR	A
Kenya				
KE18	375	NR	NR	A
Mozambique				
MO32	350	NR	NR	A
MO91	5,230,000	4.5	NR	A
MO69	632	NR	NR	A
Mali				
MA92	834	NR	1137.8	E
MA51	1580	NR	NR	E
MA57	2930	130.8	4828.4	E
MA76	2,290,000	NR	NR	E
MA31	267	NR	1.6	E
MA79	32,700,000,000	1077.6	30.2	E
Nigeria				
NI33	19,500,000,000	NR	NR	E

[a] Analysis was restricted to 12 Hepatitis B surface antigen positive women with HBV DNA >200 IU/ml who were successfully genotyped.
[b] HBV DNA (IU/ml): Concentration of hepatitis B virus deoxyribonucleic acid in international unit per millilitre detected among HBsAg positive mothers.
[c] HBeAg (S/CO): Concentration of hepatitis B extractable detected among HBsAg positive mothers in signal to cut off unity.
[d] NR: non-reactive.

Similar results were reported by Loarec and colleagues [33], which could be explained due to relatively low HBV viral loads [34]. Vertical transmission of HBV is closely linked to the maternal HBV DNA viral load [1]. With pregnant women in Mozambique having low HBV viral loads, the likelihood of transmission to newborns could be reduced, even with high HBsAg prevalence.

In our study, the majority of cord blood HBsAg positivity (92.3 %) in newborns was observed among pregnant women with an HBV viral load <200,000 IU/ml (i.e. below the recommended threshold for initiating antiviral therapy) [10,35], including 33.3 % (n = 3) in whom the viral load was <10 IU/ml. All three women with HBV viral loads <10 IU/ml, whose babies were positive for HBsAg, exhibited high HBsAg titers. This observation supports findings from other studies, indicating that low maternal HBV DNA viral loads combined with elevated HBsAg concentrations are risk factors for HBV transmission to newborns [36]. According to the 2024 global Hepatitis guidelines, all women testing positive for HBsAg, regardless of HBV DNA or HBeAg status, would be eligible for antiviral prophylaxis [4]. The current guidelines are unlikely to miss women with low HBV DNA levels (i.e <200,000 IU/ml), which was 66.6 % (8/12) among the HBsAg positive women in our study; and addresses concerns from earlier recommendation which relied on treatment based on HBV DNA thresholds, according to which a considerable proportion of women wouldn't have qualified for antiviral therapy during pregnancy. The shift toward universal prophylaxis represents a pragmatic and inclusive strategy to reduce the risk of vertical transmission, particularly in resource-limited settings.

Genotype E was the most frequently identified strain among the limited number of successfully sequenced HBV reactive women including those with HBsAg positive newborns, likely reflecting regional genotype distribution. Given the low sequencing success rate and potential confounding by viral load, this observation should be interpreted with caution. However, genotype C is often associated with a higher risk of vertical transmission due to its higher transmission efficiency and viral load [37]. Notably, West African countries (Mali and Nigeria) in our study had higher rate of cord blood HBV positivity (59.3 %–75.0 %) than the Southern African (Mozambique and South Africa; 0 %) or East African (Kenya and Ethiopia; 0 %–25.0 %). In Ghana, a study reported 16 % HBV prevalence among pregnant women, with genotype E accounting for 99 % of maternal cases and 8 % of fetal transmission cases; in Angola, HBV genotype E was detected in 7.3 % of newborns from HBsAg positive mothers [38,39]. We did not detect any antiviral resistance mutations and only one vaccine escape mutation (128 V). Consequently, existing antiviral treatments remain effective, and maintaining robust vaccination strategies would be effective for preventing HBV infection.

Limitations: We limited enrollment to healthy pregnant women without HIV who delivered at term pregnancy. Consequently, chances are that we may have underestimated HBV, as at risk groups such as women living with HIV, as well as women with adverse pregnancy outcomes such as stillbirths and preterm births, were excluded. Although the data are not necessarily generalizable, it likely provides a conservative estimate of HBsAg positivity in the mother-newborn dyads. Also, the design of the parent study only allowed for the testing of cord blood samples for HBsAg. Despite having used standardized methods for sample collection, there remains a possibility of contamination of cord blood sample with maternal blood [29], which could lead to HBsAg and HBV DNA positivity in cord blood, hence, warranting caution in inferring HBV infection of the newborn based only on testing of cord blood samples. Furthermore, the presence of HBV markers (HBsAg, HBeAg, or HBV DNA) in cord blood could be transient, and whilst indicating *in utero* exposure to HBV, it does not confirm HBV infection in the newborn. A definitive diagnosis of vertical transmission of HBV requires post-natal follow-up and testing of the infant, typically at or beyond 6 months of age, to mitigate confounding due to vaccine-induced transient antigenemia. Another limitation of this study is the lack of data on antenatal HBV screening, thereof potential biases related to prior HBV screening could not be accounted in our analysis, that may influence the interpretation of findings, particularly in countries with routine antenatal HBV screening.

In conclusion, our findings indicate the need for country-specific data to inform the public health importance of maternal HBV infection and cord blood HBsAg positivity rates to newborns. Also, the criteria for initiating antiviral treatment in pregnant women may need to be expanded to include women with lower HBV viral load, but high HBsAg titer, in settings where there is a significant burden of HBV vertical transmission. Finally, while HBsAg in cord blood warrants attention, it is not conclusive evidence of vertical transmission. Definitive diagnosis requires follow-up testing after the neonatal prophylaxis period. Prophylaxis remains the cornerstone of prevention.

CRediT authorship contribution statement

Carine Bokop: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Nisha Dhar:** Writing – review & editing, Supervision, Formal analysis, Data curation, Conceptualization. **Alane Izu:** Writing – review & editing, Formal analysis, Data curation. **Jayendrie Thaver-Kleitman:** Writing – review & editing, Methodology, Formal analysis. **Nishi Prabdial-sing:** Writing – review & editing, Methodology. **Musa Mohammed Ali:** Writing – review & editing, Investigation. **Godwin Akaba:** Writing – review & editing, Investigation. **Hellen C. Barsosio:** Writing – review & editing, Investigation. **James A. Berkley:** Writing – review & editing, Investigation. **Manisha Madhai Beck:** Writing – review & editing, Investigation. **Tolossa E. Chaka:** Writing – review & editing, Investigation. **Clare L. Cutland:** Writing – review & editing, Investigation. **Phurb Dorji:** Writing – review & editing, Investigation. **Maksuda Islam:** Writing – review & editing, Investigation. **Adama Mamby Keita:** Writing – review & editing, Investigation. **Feleke Belachew Lema:** Writing – review & editing, Investigation. **Nubwa Medugu:** Writing – review & editing, Investigation. **Stella Mwakio:** Writing – review & editing, Investigation. **Stephen Obaro:** Writing – review & editing, Investigation. **Eyinade. K. Olateju:** Writing – review & editing, Investigation. **Rani Diana Sahni:** Writing – review & editing, Investigation. **Samir K. Saha:** Writing – review & editing, Investigation. **Sridhar Santhanam:** Writing – review & editing, Investigation. **Ragunath Sharma:** Writing – review & editing, Investigation. **Betuel Sigaúque:** Writing – review & editing, Investigation. **Eric A.F. Simoes:** Writing – review & editing, Investigation. **Samba O. Sow:** Writing – review & editing, Investigation. **Milagritos D. Tapia:** Writing – review & editing, Investigation. **Balaji Veeraraghavan:** Writing – review & editing, Investigation. **Gaurav Kwatra:** Writing – review & editing, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Shabir A. Madhi:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Carine Bokop reports financial support was provided by Poliomyelitis Research Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Wits-Vaccines Infectious Diseases Analytics Research Unit and Poliomyelitis Research Foundation (#22/107) for providing funds for this research. We thank all the participants in the parent GBS-MCS study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jcv.2025.105826.

References

[1] S. Peng, Z. Wan, T. Liu, H. Zhu, Y. Du, Incidence and risk factors of intrauterine transmission among pregnant women with chronic hepatitis B virus infection, J. Clin. Gastroenterol. 53 (2019) 51–57.

[2] World Health Organization, Hepatitis B vaccines, WHO position paper, Weekly Epidemiological Record, 2009, p. 405–420.

[3] Center for Disease Control and Prevention, The ABCs of Hepatitis, US Centers for Disease Control and Prevention, 2012.

[4] Global Hepatitis Report 2024, Global Hepatitis Report 2024: Action for Access in Low- and Middle-income Countries, Geneva, 2024.

[5] World Health Organization, Combating Hepatitis B and C to Reach Elimination by 2030, World Health Organization, 2016.

[6] M. Dagnew, A. Belachew, M. Tiruneh, F. Moges, Hepatitis E virus infection among pregnant women in Africa: systematic review and meta-analysis, BMC Infect. Dis. 19 (2019) 519.

[7] World Health Organization, 91 Million Africans Infected with Hepatitis B or C, World Health Organization Africa, 2022.

[8] Y. Ren, Y. Guo, L. Feng, T. Li, Y. Du, Controversy and strategies exploration in blocking mother-to-child transmission of hepatitis B, Int. Rev. Immunol. 35 (2016) 249–259.

[9] Y. Chen, L. Wang, Y. Xu, X. Liu, S. Li, Q. Qian, B. Hu, A. Zhou, T. Chen, Y. Zhao, Role of maternal viremia and placental infection in hepatitis B virus intrauterine transmission, Microbes Infect. 15 (2013) 409–415.

[10] Jr Brown RS Jr., B.J. McMahon, A.S. Lok, J.B. Wong, A.T. Ahmed, M.A. Mouchli, Z. Wang, L.J. Prokop, M.H. Murad, K. Mohammed, Antiviral therapy in chronic hepatitis B viral infection during pregnancy: a systematic review and meta-analysis, Hepatology 63 (2016) 319–333.

[11] X. Zhao, X. Bai, Y. Xi, Intrauterine infection and mother-to-child transmission of hepatitis B virus: route and molecular mechanism, Infect. Drug Resist. 15 (2022) 1743–1751.

[12] D. Tegegne, K. Desta, B. Tegbaru, T. Tilahun, 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 (2014) 239.

[13] A. Boisson, V. Goel, M. Yotebieng, J.B. Parr, B. Fried, P. Thompson, Implementation approaches for introducing and overcoming barriers to hepatitis B birth-dose vaccine in sub-Saharan Africa, Glob. Health Sci. Pract. 10 (2022).

[14] World Health Organization, Hepatitis B Control in the South East Asia Region, World Health Organization, 2023.

[15] S.M.F. Akbar, M. Al Mahtab, F. Begum, S.A.S. Hossain, S. Sarker, A. Shrestha, M. Khan, O. Yoshida, Y. Hiasa, Implications of birth-dose vaccination against hepatitis B virus in Southeast Asia, Vaccines 9 (2021).

[16] G. Kwatra, A. Izu, C. Cutland, G. Akaba, M.M. Ali, Z. Ahmed, M.M. Beck, H. C. Barsosio, J.A. Berkley, T.E. Chaka, A. Cossa, S. Chakraborty, N. Dhar, P. Dorji, M. Islam, A.M. Keita, S. Mwakio, S. Mwarumba, N. Medugu, H. Mucavele, V. Mabombo, S. Obaro, B. Sigaúque, S.O. Sow, S.K. Saha, S. Santhanam, R. Sharma, E. Simoes, R.D. Sahni, M.D. Tapia, B. Veeraraghavan, S.A. Madhi, Prevalence of group B Streptococcus colonisation in mother-newborn dyads in low-income and middle-income south Asian and African countries: a prospective, observational study, Lancet Microbe 5 (2024) 100897.

[17] L. Breakwell, C. Tevi-Benissan, L. Childs, R. Mihigo, R. Tohme, The status of hepatitis B control in the African region, Pan Afr. Med. J. 27 (2017) 17.

[18] C.Q. Pan, Z.P. Duan, K.R. Bhamidimarri, H.B. Zou, X.F. Liang, J. Li, M.J. Tong, An algorithm for risk assessment and intervention of mother to child transmission of hepatitis B virus, Clin. Gastroenterol. Hepatol. 10 (2012) 452–459.

[19] N. Saitou, M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees, Mol. Biol. Evol. 4 (1987) 406–425.

[20] W. Li, W. Wei, F. Hou, H. Xu, X. Cui, The integration model of hepatitis B virus genome in hepatocellular carcinoma cells based on high-throughput long-read sequencing, Genomics 114 (2022) 23–30.

[21] M. Bittaye, P. Idoko, B.A. Ekele, S.A. Obed, O. Nyan, Hepatitis B virus sero-prevalence amongst pregnant women in the Gambia, BMC Infect. Dis. 19 (2019) 259.

[22] B.A. Dortey, E.A. Anaba, A.T. Lassey, N.K.R. Damale, E.T. Maya, Seroprevalence of Hepatitis B virus infection and associated factors among pregnant women at Korle-Bu Teaching Hospital, Ghana, PLoS One 15 (2020) e0232208.

[23] J. Nankya-Mutyoba, J. Aizire, F. Makumbi, L. Atuyambe, P. Ocama, G.D. Kirk, Correlates of hepatitis B awareness and disease-specific knowledge among pregnant women in Northern and Central Uganda: a cross-sectional study, Hepatol. Med. Policy 3 (2018) 14.

[24] M. Shamsuzzaman, P. Singhasivanon, J. Kaewkungwal, S. Lawpoolsri, P. Tangkijvanich, R.V. Gibbons, M. Rahman, A.S. Alamgir, M.A. Mahtab, Hepatitis B among pregnant women attending health care facilities in rural Bangladesh, Southeast Asian J. Trop. Med. Public Health 42 (2011) 1410–1413.

[25] S. Giri, S. Sahoo, S. Angadi, S. Afzalpurkar, S. Sundaram, S. Bhrugumalla, Seroprevalence of hepatitis B virus among pregnant women in India: a systematic review and meta-analysis, J. Clin. Exp. Hepatol. 12 (2022) 1408–1419.

[26] G. Da Villa, A. Andjaparidze, M. Cauletti, E. Franco, M. Roggendorf, A. Sepe, L. Zaratti, Viral hepatitis in the Bhutanese population: preliminary results of a seroepidemiological investigation, Res. Virol. 148 (1997) 115–117.

[27] United Nations International Children's Emergency Fund, UNICEF Data: Monitoring the Situation of Children and Women, 2024.

[28] World Health Organization, WHO Guidelines on Hepatitis B and C Testing, World Health Organization, 2017.

[29] K. Geffert, T.G. Maponga, S. Henerico, W. Preiser, S. Mongella, A. Stich, S. Kalluvya, A. Mueller, C. Kasang, Prevalence of chronic HBV infection in pregnant woman attending antenatal care in a tertiary hospital in Mwanza, Tanzania: a cross-sectional study, BMC Infect. Dis. 20 (2020) 395.

[30] O.A. Olaleye, O. Kuti, N.O. Makinde, A.O. Ujah, O.A. Olaleye, O.O. Badejoko, A. A. Akintayo, R. Audu, Perinatal transmission of hepatitis B virus infection in Ile-Ife, South Western, Nigeria, J. Neonatal Perinat. Med. 6 (2013) 231–236.

[31] J. Liu, B. Xu, T. Chen, J. Chen, J. Feng, C. Xu, L. Liu, Y. Hu, Y.H. Zhou, Presence of hepatitis B virus markers in umbilical cord blood: exposure to or infection with the virus? Dig. Liver Dis. 51 (2019) 864–869.

[32] R. Mistik, B. Baran, The transfer of hepatitis B virus (HBV) from HBsAg(+) mothers to their babies, Mikrobiyol. Bul. 27 (1993) 143–146.

[33] A. Loarec, A. Nguyen, L. Molfino, M. Chissano, N. Madeira, B. Rusch, N. Staderini, A. Couto, I. Ciglenecki, N.T. Antabak, Prevention of mother-to-child transmission of hepatitis B virus in antenatal care and maternity services, Mozambique, Bull. World Health Organ. 100 (2021) 60–69.

[34] G. Wandeler, K. Musukuma, S. Zürcher, M.J. Vinikoor, J. Llenas-García, M.M. Aly, L. Mulenga, B.H. Chi, J. Ehmer, M.A. Hobbins, C. Bolton-Moore, C.J. Hoffmann, M. Egger, A. IeDEA-Southern, Hepatitis B infection, viral load and resistance in HIV-infected patients in Mozambique and Zambia, PLoS ONE 11 (2016) 0152043.

[35] European Association for the Study of the Liver, EASL, Electronic address: easloffice@easloffice.eu, & European Association for the Study of the Liver clinical practice guidelines on the management of hepatitis B virus infection, J. Hepatol., 67, 2017, pp. 370–398.

[36] Z.X. Chen, G.F. Gu, Z.L. Bian, W.H. Cai, Y. Shen, Y.L. Hao, S. Zhang, J.G. Shao, G. Qin, Clinical course and perinatal transmission of chronic hepatitis B during pregnancy: a real-world prospective cohort study, J. Infect. 75 (2017) 146–154.

[37] J. Li, J. Li, S. Chen, W. Xu, J. Zhang, S. Tong, Clinical isolates of hepatitis B virus genotype C have higher in vitro transmission efficiency than genotype B isolates, J. Med. Virol. 95 (2023) e28879.

[38] D. Candotti, K. Danso, J.P. Allain, Maternofetal transmission of hepatitis B virus genotype E in Ghana, west Africa, J. Gen. Virol. 88 (2007) 2686–2695.

[39] D. Oliveira, J. Piedade, R. Castro, À. Lopes, M.D.R. Martins, F. Pereira, Evaluating vertical transmission of sexually transmitted infections to newborns, J. Infect. Dev. Ctries. 15 (2021) 1547–1550.