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

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Summary

Background In sub-Saharan Africa, administration of hepatitis B virus (HBV) birth-dose vaccines remains suboptimal. Evidence is scarce on whether African countries should focus on increasing vaccine coverage or developing strategies incorporating additional measures, such as peripartum antiviral prophylaxis to pregnant women at high risk. To better inform decision makers, we estimated the residual risk of mother-to-child transmission despite HBV birth-dose vaccine in Cameroon.

Methods We did a single-centre, longitudinal observational study. Pregnant women were systematically screened for HBV surface antigen (HBsAg) at Tokombéré District Hospital (Tokombéré district, Cameroon). Children born to HBsAg-positive mothers in 2009–16 who received the HBV birth-dose vaccine and three subsequent doses of pentavalent vaccine at 6, 10, and 14 weeks were followed up prospectively in 2015–17. In children, capillary blood was obtained for HBsAg rapid test and dried blood spots to quantify HBV DNA concentrations. Venous blood was also collected from HBsAg-positive children. Mother-to-child transmission was confirmed by whole-genome sequencing.

Findings Between Jan 31, 2009, and Dec 31, 2016, 22 243 (66·8%) of 33 309 pregnant women accepted antenatal HBV screening, of whom 3901 (17·5%) were HBsAg positive. 2004 (51·4%) of 3901 children who were born to HBsAg-positive mothers received the HBV birth-dose vaccine, of whom 1800 (89·8%) also completed the three-dose pentavalent vaccine. In total, the current analysis included 607 children who had a follow-up serosurvey. The prevalence of HBsAg was 5·6% in children who received the birth-dose vaccine in less than 24 h, 7·0% in those who received it 24–47 h after birth, and 16·7% in those who received it 48–96 h after birth (p_{trend}=0·083). 35 (89·7%) of 39 infected children were born to mothers positive for HBV e antigen with high HBV DNA of 5·3 $\log_{10}$ IU/mL or more. Whole-genome sequencing of HBV in infected mother-child pairs confirmed high identity proportions of 99·97–100%.

Interpretation We documented a substantial risk of mother-to-child transmission despite timely administration of the HBV birth-dose vaccine within 24 h after birth. To reach WHO's elimination targets, peripartum antiviral prophylaxis might be required in parts of Africa, in addition to increasing coverage of the HBV birth-dose vaccine.

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Introduction

Worldwide, 296 million people are chronically infected with hepatitis B virus (HBV) and approximately 820 000 die annually due to HBV-related cirrhosis or hepatocellular carcinoma.[1] WHO aims to eliminate HBV infection as a public health threat by 2030, and one of the goals is to attain a 0·1% prevalence of hepatitis B surface antigen (HBsAg) in children aged 5 years.[1] Prevention of perinatal mother-to-child transmission of HBV is essential for eliminating this infection, because this transmission mode is an important risk factor for chronic HBV infection and HBV-related chronic liver diseases, compared with horizontal transmission later in life.[2]

Since 2009, WHO recommends that all infants, irrespective of maternal HBV serostatus, should receive a series of 3–4 doses of HBV vaccine starting immediately after birth, preferably within 24 h, to prevent HBV mother-to-child transmission.[3] This HBV birth-dose vaccine has been successfully implemented, particularly in Asia.[4] However, HBV birth-dose vaccines might not prevent all mother-to-child transmission, and the risk might persist in infants born to HBV-infected mothers who have high viral replication.[5] In resource-rich Asian countries, hepatitis B immune globulin has been given to these infants, in addition to the HBV birth-dose vaccine.[6] Moreover, to further eliminate the residual risk of mother-to-child transmission despite the infant immunoprophylaxis, WHO and other professional societies now recommend peripartum antiviral prophylaxis using nucleoside or nucleotide analogues to

Research in context

Evidence before this study

Although sub-Saharan Africa bears the highest burden of hepatitis B virus (HBV) infection worldwide, measures to prevent mother-to-child transmission have been poorly implemented. By 2021, only 14 (30%) of 47 countries in this region had introduced HBV birth-dose vaccine as per WHO's recommendation. Consequently, the risk of mother-to-child transmission remains poorly estimated in African children who complete a series of three to four doses of HBV vaccine beginning immediately after birth. We searched PubMed for articles published from database inception up to April 15, 2021, in any language, using the following terms: "hepatitis B" AND "mother-to-child transmission" AND "sub-Saharan Africa". One systematic review by Keane and colleagues identified three longitudinal studies in sub-Saharan Africa assessing the risk of mother-to-child transmission in children who have received HBV birth-dose vaccine. These studies were limited by the risk of bias with a small sample size. One medium-sized study in Côte d'Ivoire by Ekra and colleagues found that nine (5·8%) of 156 infants born to HBV-infected mothers became infected despite receiving HBV birth-dose vaccine. However, this study did not examine maternal HBV DNA concentrations, a major determinant of mother-to-child transmission. Moreover, the study excluded children who received HBV birth-dose vaccine beyond the recommended period of 24 h after birth; the evaluation of mother-to-child transmission in this group is relevant, because uptake of institutional delivery remains low in this region.

Added value of this study

In 607 children born to HBV-infected mothers who completed the four-dose vaccination at weeks 0, 6, 10, and 14 in northern Cameroon, the risk of hepatitis B surface antigen (HBsAg) positivity tended to increase with a delay in the timing of the first dose: 5·6% in those receiving the vaccine less than 24 h after birth, 7·0% in those receiving it 24–47 h after birth, and 16·7% in those receiving it 48–96 h after birth (p_{trend}=0·08). Following a cross-classification by both maternal HBV e antigen (HBeAg; positive or negative) and maternal HBV DNA concentrations (high [≥5·3 $\log_{10}$ IU/mL] or low [<5·3 $\log_{10}$ IU/mL]), the risk of HBsAg positivity in children who had a timely HBV birth-dose vaccine (<24 h) was 32·4% from HBeAg-positive mothers with high viraemia, 0% from HBeAg-positive mothers with low viraemia, 0% from HBeAg-negative mothers with high viraemia, and 0·3% from HBeAg-negative mothers with low viraemia. Whole-genome sequencing of HBV strains revealed that 96·2% of infected mother-child pairs showed identical sequences of 99·97–100%, strongly suggesting that children acquired HBV infection from their mothers.

Implications of all the available evidence

This study indicated the importance of timely administration of HBV birth-dose vaccine within 24 h of birth in a real-life field condition in sub-Saharan Africa. However, a substantial risk persists despite the timely HBV birth-dose vaccine, particularly in infants born to HBeAg-positive mothers with a high viral load. HBV birth-dose vaccine alone is unlikely to be sufficient to attain WHO's HBV elimination goal of 0·1% prevalence in children aged 5 years. An additional strategy, such as antenatal HBV screening and peripartum antiviral prophylaxis, might be required in parts of Africa in addition to efforts to increase the immunisation coverage.

HBV-infected pregnant women with high viral loads (≥5·3 $\log_{10}$ IU/mL).[3] However, these recommendations were mostly based on Asian studies.[5,7]

Sub-Saharan Africa has a high prevalence of HBsAg, exceeding 8% in the general population.[8] Despite this heavy burden, interventions to prevent HBV mother-to-child transmission have been poorly implemented.[9] Most countries schedule HBV vaccine starting only at 6–8 weeks of age as a pentavalent vaccine.[10] Even in a few countries that have integrated the HBV birth-dose vaccine into the infant immunisation programme, its timely administration within 24 h after birth is not well performed because of a low institutional birth rate and a lack of coordination within a health facility.[11] Consequently, the annual number of infants perinatally infected with HBV is estimated to be twice the number of incident paediatric HIV infections in sub-Saharan Africa.[12] Given the limited health-care resources in this region, it is crucial to determine whether African countries should primarily focus their efforts on increasing the HBV birth-dose vaccine coverage or develop a strategy, similar to Asia, incorporating both

HBV birth-dose vaccine and additional interventions including hepatitis B immune globulin or peripartum antivirals. However, a paucity of African data exists for the residual risk of mother-to-child transmission in infants who received the HBV birth-dose vaccine.[12]

Cameroon has high HBsAg prevalence in pregnant women (9·8%).[13] Since 2005, three doses of pentavalent HBV vaccine have been scheduled at 6, 10, and 14 weeks of life (ie, HepB3) as part of the national immunisation programme; the HBV birth-dose vaccine, however, has not yet been introduced. In 2007, a pilot project for antenatal HBV screening and selective HBV birth-dose vaccine administration was launched in Tokombéré, a rural health district in the far north region in Cameroon, where about 150 000 people live.[14] A cohort of children born to HBsAg-positive women between January, 2009, and December, 2016, was prospectively followed-up for their HBV status between May, 2015, and August, 2017. In this study, we aim to estimate the residual risk of mother-to-child transmission despite the completion of a series of HBV vaccination including the HBV birth-dose vaccine, evaluate the performance of maternal HBV

markers during pregnancy to predict HBsAg positivity in the child, and compare HBV strains in infected mother-child pairs by whole-genome sequencing.

Methods

Study design and participants

We did a single-centre, longitudinal observational study. In 2009–16, all women attending antenatal care, whether or not they had already been tested for these infections, were invited for HBV and HIV screening at the Tokombéré District Hospital, the only hospital in the Tokombéré district, Cameroon.

Capillary blood was collected from pregnant women for rapid tests: VIKIA HBsAg (bioMérieux; Craponne, France) and Alere Determine HIV-1/2 (Abbott; Abbott Park, IL, USA). The results were immediately given to the women onsite and post-test counselling was provided at the same time. HBsAg-positive women underwent a standardised questionnaire and provided venous blood. Neonates born to these mothers in Tokombéré District Hospital were given an HBV birth-dose vaccine (Engerix-B Pediatric; GlaxoSmithKline; Brentford, UK) as soon as possible after their birth. Those born at home or at primary health centres were vaccinated by the immunisation officer who made outreach vaccination trips whenever a notification was made by local community health workers on the day of childbirth. In circumstances where its timely administration (<24 h) was not possible, particularly in the evenings or during weekends when immunisation staff were absent, delayed administration was allowed up to 3 days after birth. Other vaccines, including HepB3 (Shan 5; Shantha, India), were administered according to the national recommendation.[15]

Children born to HBsAg-positive mothers in 2009–16 were followed-up in 2015–17. Children were eligible if they had documented evidence of completion of both HBV birth-dose vaccine and HepB3 based on the vaccination card or registry and if the maternal sample at the time of pregnancy was available. Children older than the recommended age for mother-to-child transmission assessment (7–12 months)[3] were also included because the risk of horizontal transmission is negligible in those who have completed 3–4 doses of infant vaccination.[16] Community health workers invited all eligible children to Tokombéré District Hospital. For those who could not afford to travel, the mobile team made a home visit. Capillary blood was obtained for HBsAg rapid test and dried blood spots to quantify HBV DNA concentrations. Venous blood was also collected from HBsAg-positive children. All participants provided written informed consent. This study was approved by the National Ethics Committee (2014/04/443/CE/CNERSH/SP).

Laboratory procedures

Maternal serum samples were locally tested for hepatitis B e antigen (HBeAg; Monolisa; BioRad; Marnes-la-Coquette, France). Serum and plasma from HBsAg-positive pregnant women, serum from HBsAg-positive children, and dried blood spots from all children were kept frozen at –20°C and shipped to France for additional tests. Maternal serum samples were used to confirm positive HBsAg (ARCHITECT; Abbott) and positive HBeAg (ETI-EBK PLUS; Diasorin; Saluggia, Italy), and tested for HBV core-related antigen (HBcrAg; LUMIPULSE G600II; Fujirebio Europe; Gent, Belgium). Maternal antibody to hepatitis D virus (anti-HDV) was evaluated (ETI-AB-DELTAK-2; Diasorin) only when their children were HBsAg-positive in order to document HDV mother-to-child transmission. In HBsAg-negative children, hepatitis B surface antibody (anti-HBs) was detected using dried blood spots (appendix p 2).

HBV DNA concentrations were quantified by real-time transcription-mediated amplification (Aptima HBV Quant Assay; Hologic; Marlborough, MA, USA) using plasma from HBsAg-positive pregnant women and dried blood spots from all children.[17] HBV DNA was extracted using the NucliSens easyMAG system (bioMérieux). Full-length pol gene was amplified by nested PCR,[18] and sequenced by Sanger method. MinION long read sequencing of HBV was done as previously described,[19] except that full-length amplicons were barcoded (EXP-PBC001; Oxford Nanopore Technology [ONT]; Oxford, UK), pooled and sequenced on Flongle flow cells (SQK-LSK109 kit and FLO-FLG106, R9.4.1; ONT; Oxford, UK). A Maximum Likelihood Phylogenetic Reconstruction was then done with curated genome sequences available from GenBank. The multiple sequence alignment was built using MUSCLE (version 10), and phylogenetic reconstruction conducted in RAxML (version 8) program.[20] A circular tree was produced with the iTOL viewer (version 5).[21]

Statistical analysis

Mother-child pairs without final child outcome data were excluded from the analysis. To identify risk factors for child HBsAg positivity, a χ^2 test was used for unordered categorical variables and a test for linear trend was done by fitting logistic regression for ordered categorical variables. Directed acyclic graph was used to select covariates that should be included in a multivariable logistic regression (appendix p 3). To better elucidate the independent role of maternal HBeAg and maternal HBV DNA concentrations, child outcomes were stratified by maternal HBeAg (positive or negative), viral loads (high [$\geq 5\cdot3$ $\log_{10}$ IU/mL] or low [$<5\cdot3$ $\log_{10}$ IU/mL]), and timing of HBV birth-dose vaccine (timely [<24 h] or delayed [24–96 h]). Performance of maternal HBV markers during pregnancy to predict child HBV infection was assessed using the area under the receiver operating characteristic curve (AUROC) in children who had a timely HBV birth-dose vaccine. A subgroup analysis was done by restricting to those younger than 24 months.

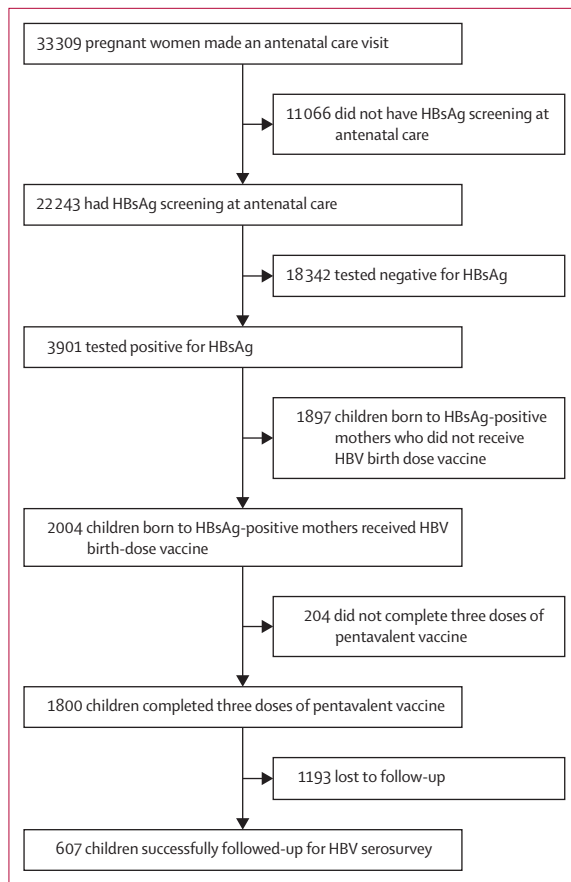All analyses were done using STATA (version 14.0).

See **Online** for appendix

Figure: Flowchart of study participants
HBV=hepatitis B virus. HBsAg=HBV surface antigen.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Jan 31, 2009, and Dec 31, 2016, 33 309 pregnant women visited Tokombéré District Hospital, of whom 22 243 (66·8%) were screened for HBsAg and of whom 3901 (17·5%) were HBsAg positive. 2004 (51·4%) of 3901 neonates who were born to HBsAg-positive mothers received the HBV birth-dose vaccine, of whom 1800 (89·8%) also completed the three-dose pentavalent vaccine (figure). The current analysis included 607 children who had a follow-up serosurvey, including five pairs of twins; table 1 summarises their characteristics. At the time of the antenatal screening, the median age of their mothers was 24 years (IQR 20–30). Serum HBeAg was detected in 128 (21·4%) of 597 HBsAg-positive women, HBcrAg in 369 (62·2%) of 593, and HBV DNA in 346 (58·2%) of 594. HBeAg prevalence in pregnant women decreased with an increase in their age: 50 (37·6%) of 133 mothers aged 15–19 years, 28 (15·8%) of 177 aged 20–24, 44 (19·5%) of

226 aged 25–34, and five (8·5%) of 59 aged 35 years or older (p_{trend}<0·0001). Only one (0·2%) of 605 women was co-infected with HIV. None carried anti-HDV. 383 (63·1%) of 607 children were born at Tokombéré District Hospital, 164 (27·0%) at primary health centres, and 60 (9·9%) at home. HBV birth-dose vaccine administration was timely (<24 h) in 426 (70·2%) of 607 children and delayed (24–96 h) in 181 (29·8%). The appendix (p 4) summarises the characteristics of children born to HBsAg-positive mothers by the timeliness of receiving the HBV birth-dose vaccine.

Samples from children were obtained at a median age of 36 months (IQR 20–53). 39 (6·4%) of 607 children were positive for HBsAg and 36 (5·9%) for HBV DNA. 34 (5·6%) children were positive for both markers, 566 (93·2%) were negative for both, five (0·8%) were HBsAg positive with undetectable viral load, and two (0·3%) were HBsAg negative with detectable HBV DNA.

Table 1 shows the results of the crude analysis of risk factors for child HBsAg positivity. The risk of child HBsAg was significantly higher when their mothers were young (p=0·038), had HBeAg (p<0·0001), had high HBcrAg concentrations (p<0·0001), and had high HBV DNA concentrations (p<0·0001). 35 (89·7%) of 39 infected children were born to mothers positive for HBeAg with high HBV DNA of 5·3 $\log_{10}$ IU/mL or more. Although the association was not statistically significant, a trend was observed between early HBV birth-dose vaccine administration and the risk of child HBsAg positivity; the risk was 5·6% in children who received the birth-dose vaccine in less than 24 h, 7·0% in 24–47 h, and 16·7% in 48–96 h (p_{trend}=0·083). The risk of child HBsAg tended to be higher with an increase in their age at assessment. The appendix (p 5) presents the odds ratios (ORs) of factors associated with child HBsAg-positivity adjusted for covariates selected by the directed acyclic graph. The following variables were found to have a significant association with child HBV infection: born to young mothers, positive HBeAg, high HBcrAg concentrations of 5·3 $\log_{10}$ U/mL or more, and high HBV DNA concentration of 5·3 $\log_{10}$ IU/mL or more (appendix p 5).

Table 2 presents the child outcomes stratified by maternal HBeAg, maternal viral loads, and the timing of HBV birth-dose vaccine. In the timely HBV birth-dose vaccine group, the risks of HBsAg positivity were 32·4% (23 of 71) from HBeAg-positive mothers with high viraemia (≥5·3 $\log_{10}$ IU/mL), 0% (none of eight) from HBeAg-positive mothers with low viraemia (<5·3 $\log_{10}$ IU/mL), 0% (none of 11) from HBeAg-negative mothers with high viraemia, and 0·3% (one of 338) from HBeAg-negative mothers with low viraemia. In the delayed HBV birth-dose vaccine group, the risks were 30·8% (12 of 39) from HBeAg-positive mothers with high viraemia, 22·2% (two of nine) from HBeAg-positive mothers with low viraemia, and 0·8% (one of 126) from

HBeAg-negative mothers with low viraemia. No children were born to HBeAg-negative mothers with high viraemia in this group.

Table 3 shows the performance of maternal HBV markers during pregnancy to predict the risk of child HBsAg positivity despite the timely HBV birth-dose vaccine in 414 children with complete maternal virological assessment. The AUROC of HBV DNA was 0·9517 (95% CI 0·9253–0·9779), of HBcrAg was 0·9329 (0·9006–0·9652), and of HBeAg was 0·9074 (0·8629–0·9517). By applying the HBV DNA threshold recommended by the international guidelines for peripartum antiviral prophylaxis ($\geq 5\cdot3$ $\log_{10}$ IU/mL),[3] the sensitivity of maternal HBV DNA during pregnancy to predict the infant immunoprophylaxis failure was 95·8% and specificity was 84·9%. By using the HBcrAg cutoff ($\geq 5\cdot3$ $\log_{10}$ U/mL) corresponding to high HBV DNA concentrations of 5·3 $\log_{10}$ IU/mL or more,[22] the sensitivity of HBcrAg was 91·7% and specificity was 84·9%. Maternal HBeAg had a comparative sensitivity of 95·8% and specificity of 85·6%. Similar results were observed when restricting the analysis to 133 children who had HBsAg assessment before 24 months of age (appendix p 6).

Of the 39 pairs of HBsAg-positive children and their mothers, the whole-genome sequencing was successfully achieved in 26 pairs, one isolated mother, and one isolated child (table 4; GenBank accession numbers: MZ312033–MZ312084). All the strains belonged to genotype E, except one mother and her child, both infected by genotype D. An HBsAg escape mutation was found in only one mother-child pair with a substitution at codon 129 (Gln129His). No other mutation was found, except a serine at HBsAg position 140 identified in all the genotype E strains. The phylogenetic analysis showed an important homology in mother-child pairs (appendix pp 7–8). 25 (96·2%) of 26 mother-child pairs showed identical sequences of 99·97–100% in the whole genome. In one mother-child pair, the percentage of identity was 99·34%; this percentage corresponds to a difference of 21 base pairs, suggesting that the child might have horizontally acquired HBV rather than through mother-to-child transmission. The sequences obtained from unrelated children and mothers were not closely related to each other (data not shown). Full-length pol sequences obtained by the Sanger method were consistent with the results obtained by whole-genome sequencing by MinION (data not shown).

Anti-HBs was tested using dried blood spots in 564 HBsAg-negative children (limit of detection 30 mIU/mL). Anti-HBs was detected in 358 (63·5%) of 564 children, with a prevalence varying according to their age: 47 (78·3%) of 60 children younger than 12 months, 73 (67·6%) of 108 aged 12–23 months, 89 (74·8%) of 119 aged 24–35 months, 72 (69·9%) of 103 aged 36–47 months, 40 (47·1%) of 85 aged 48–59 months, and 37 (41·6%) of 89 children 60 months or older ($p_{trend} < 0\cdot0001$).

	All children (n=607)	HBsAg-negative children (n=568)	HBsAg-positive children (n=39)	p value†
Maternal age (years)				
15–19	135 (22·3%)	122/135 (90·4%)	13/135 (9·6%)	0·038‡
20–24	178 (29·4%)	167/178 (93·8%)	11/178 (6·2%)	..
25–34	230 (38·0%)	217/230 (94·4%)	13/230 (5·6%)	..
≥35	62 (10·3%)	61/62 (98·4%)	1/62 (1·6%)	..
Maternal HBeAg status				
Negative	469 (78·6%)	467/469 (99·6%)	2/469 (0·4%)	<0·0001
Positive	128 (21·4%)	91/128 (71·1%)	37/128 (28·9%)	..
Maternal HBcrAg (log₁₀ U/mL)				
Undetectable	224 (37·8%)	224/224 (100%)	0	<0·0001‡
3·0–3·9	174 (29·3%)	172/174 (98·8%)	2/174 (1·2%)	..
4·0–4·9	53 (8·9%)	52/53 (98·1%)	1/53 (1·9%)	..
5·0–5·9	27 (4·6%)	26/27 (96·3%)	1/27 (3·7%)	..
6·0–6·9	10 (1·7%)	9/10 (90·0%)	1/10 (10·0%)	..
7·0–7·9	53 (8·9%)	39/53 (73·6%)	14/53 (26·4%)	..
≥8·0	52 (8·8%)	32/52 (61·5%)	20/52 (38·5%)	..
Maternal HBV DNA (log₁₀ IU/mL)				
Undetectable	248 (41·8%)	247/248 (99·6%)	1/248 (0·4%)	<0·0001‡
1·0–1·9	47 (7·9%)	47/47 (100%)	0	..
2·0–2·9	87 (14·7%)	86/87 (98·9%)	1/87 (1·1%)	..
3·0–3·9	68 (11·4%)	66/68 (97·1%)	2/68 (2·9%)	..
4·0–4·9	21 (3·5%)	21/21 (100%)	0	..
5·0–5·9	6 (1·0%)	6/6 (100%)	0	..
6·0–6·9	9 (1·5%)	8/9 (88·9%)	1/9 (11·1%)	..
7·0–7·9	24 (4·0%)	22/24 (91·7%)	2/24 (8·3%)	..
8·0–8·9	77 (13·0%)	46/77 (59·7%)	31/77 (40·3%)	..
≥9·0	7 (1·2%)	6/7 (85·7%)	1/7 (14·3%)	..
Birthplace				
Tokombéré district hospital	383 (63·1%)	358/383 (93·5%)	25/383 (6·5%)	0·97
Primary health centres	164 (27·0%)	154/164 (93·9%)	10/164 (6·1%)	..
Home	60 (9·9%)	56/60 (93·3%)	4/60 (6·7%)	..
Time of first dose of HBV vaccine (h after birth)				
<24	426 (70·2%)	402/426 (94·4%)	24/426 (5·6%)	0·083‡
24–47	157 (25·9%)	146/157 (93·0%)	11/157 (7·0%)	..
48–96	24 (3·9%)	20/24 (83·3%)	4/24 (16·7%)	..
Child age (months)				
<12	61 (10·0%)	60/61 (98·4%)	1/61 (1·6%)	0·031‡
12–23	117 (19·3%)	110/117 (94·0%)	7/117 (6·0%)	..
24–35	124 (20·4%)	119/124 (96·0%)	5/124 (4·0%)	..
36–47	113 (18·6%)	104/113 (92·0%)	9/113 (8·0%)	..
48–59	92 (15·2%)	85/92 (92·4%)	7/92 (7·6%)	..
60–95	100 (16·5%)	90/100 (90·0%)	10/100 (10·0%)	..

Data are n (%) or n/N (%), unless otherwise specified. HBsAg=HBV surface antigen. HBeAg=HBV e antigen. HBcrAg=HBV core-related antigen. HBV=hepatitis B virus. *Study included 607 mother-child pairs. Deviations from the total are the result of missing data for maternal age (n=2), HBeAg (n=10), HBcrAg (n=14), and HBV DNA (n=13). †To identify risk factors for child HBsAg positivity, a χ^2 test was used to obtain p values for unordered categorical variables and a test was done for linear trend by fitting logistic regression for ordered categorical variables. ‡Test for linear trend was done using logistic regression.

Table 1: Characteristics of 607 children born to HBsAg-positive mothers and the crude analysis of risk factors for child HBsAg positivity*

Discussion

In a real-life African setting in north Cameroon, where HBV genotype E is predominant, we found a substantial

	Maternal high viraemia (≥5·3 log₁₀ IU/mL)		Maternal low viraemia (<5·3 log₁₀ IU/mL)		Total	
	Total number of children	HBsAg-positive children	Total number of children	HBsAg-positive children	Total number of children	HBsAg-positive children
Children with a timely HBV birth-dose vaccine within 24 h (n=420)						
Maternal serum HBeAg						
Positive	71	23 (32·4%)	8	0	79	23 (29·1%)
Negative	11	0	330	1 (0·3%)	341	1 (0·3%)
Total	82	23 (28·1%)	338	1 (0·3%)	420	24 (5·7%)
Children with a delayed HBV birth-dose vaccine within 24–72 h (n=174)						
Maternal serum HBeAg						
Positive	39	12 (30·8%)	9	2 (22·2%)	48	14 (29·2%)
Negative	0	NA	126	1 (0·8%)	126	1 (0·8%)
Total	39	12 (30·8%)	135	3 (2·2%)	174	15 (8·6%)

Data are n or n (%). HBsAg=HBV surface antigen. HBeAg=HBV e antigen. HBV=hepatitis B virus. NA=not applicable.

Table 2: Risk of HBsAg positivity in children by maternal HBeAg status, maternal viral load, and timing of hepatitis B birth-dose vaccine (n=594)

	Quantification of HBV DNA	Quantification of HBcrAg	Detection of HBeAg
AUROC	0·9517 (0·9253–0·9779)	0·9329 (0·9006–0·9652)	0·9074 (0·8629–0·9517)
p value (vs the AUROC of HBV DNA)	NA	0·19	0·0011
p value (vs the AUROC of HBcrAg)	0·19	NA	0·23
Optimal cutoff	5·3 log IU/mL*	5·3 log U/mL†	Qualitative
Sensitivity	95·8% (78·9–99·9)	91·7% (73·0–99·0)	95·8% (78·9–99·9)
Specificity	84·9% (80·9–88·3)	84·9% (80·9–88·3)	85·6% (81·8–89·0)
Positive predictive value	28·0% (18·7–39·1)	27·2% (17·9–38·2)	29·1% (19·4–40·4)
Negative predictive value	99·7% (98·3–100)	99·4% (97·8–99·9)	99·7% (98·3–100)
Positive likelihood ratio	6·3 (4·9–8·1)	6·1 (4·7–7·9)	6·7 (5·2–8·6)
Negative likelihood ratio	0·05 (0·01–0·34)	0·10 (0·03–0·37)	0·05 (0·01–0·33)

Data in parentheses are 95% CIs. AUROC=area under the receiver operating characteristic curve. HBV=hepatitis B virus. HBcrAg=HBV core-related antigen. HBeAg=HBV e antigen. HBsAg=HBV surface antigen. NA=not applicable. *This cutoff (≥5·3 log₁₀ IU/mL) is recommended by the European Association for the Study of the Liver, the American Association for the Study of Liver Diseases, and WHO to consider providing antiviral prophylaxis during pregnancy. †This HBcrAg cutoff (≥5·3 log₁₀ U/mL) was found to be the most optimal to diagnose HBV DNA concentrations of ≥5·3 log₁₀ IU/mL in a meta-analysis of the individual participant data.[22]

Table 3: Diagnostic performance of maternal HBV markers (HBV DNA, HBcrAg, and HBeAg) during pregnancy to predict HBsAg positivity in children who had a timely HBV birth-dose vaccine (n=414)

residual risk of 6·4% for HBsAg positivity in a large cohort of children born to HBsAg-positive mothers and who completed both the HBV birth-dose vaccine and HepB3. Maternal HBV DNA, HBcrAg, and HBeAg all had a similarly high predictive performance for child HBsAg positivity; nevertheless, a few children born to low-risk mothers (HBeAg negative and low viraemia) were still infected. Phylogenetic analysis showed that immune escape mutants were uncommon and that there was substantial homology in the mother-child pairs' whole-genome sequences, except for one pair, suggesting that the vast majority of these children might have been infected by their mothers.

Although the association was not significant, we found a tendency that earlier HBV birth-dose vaccine might better prevent mother-to-child transmission than delayed HBV birth-dose vaccine. This finding is in line with previous studies,[23,24] and also adds emphasis to WHO's recommendations of timely HBV vaccination within 24 h after birth.[3] However, despite timely administration, 5·6% of children were still infected. This finding is very similar to those reported by Ekra and colleagues of 5·8% in children born to HBsAg-positive mothers in Côte d'Ivoire who received a timely HBV birth-dose vaccine followed by two additional doses at weeks 6 and 14).[25] These findings suggest that HBV birth-dose vaccine alone is unlikely to be sufficient to eliminate HBV mother-to-child transmission in Africa. Assuming an 8·0% prevalence of HBsAg in pregnant women in sub-Saharan Africa,[8] the prevalence in children would not reach WHO's 0·1% goal,[1] even with 100% uptake of a timely HBV birth-dose vaccine in those born to HBsAg-positive mothers (8·0%×5·6%=0·4%). As an additional preventive measure, hepatitis B immune globulin is not adapted to the African context given its limited availability, high cost, and concerns for safety.[3,9] Alternatively, the integration of peripartum antiviral prophylaxis using nucleotide analogues should be seriously considered, as recently recommended.[3]

The next question would be, how effectively can physicians identify HBsAg-positive pregnant women who should receive peripartum antiviral therapy? On the basis of a systematic review,[5] WHO made a recommendation to quantify HBV DNA concentrations following a HBsAg-positive test to select women with high viral loads of 5·3 log₁₀ IU/mL or more for antiviral prophylaxis. As an inexpensive and reliable alternative, WHO also made a conditional recommendation to provide antivirals to HBeAg-positive women in areas where access to HBV DNA testing is limited.[3] In our study, most women whose children were vertically infected had high HBV DNA of 5·3 log₁₀ IU/mL or more (35 [89·7%] of 39) or were positive for HBeAg (37 [94·9%] of 39), supporting WHO's recommendations.[3] However, these recommendations are not perfect. In our cohort, four women with low viraemia of less than 5·3 log₁₀ IU/mL had HBsAg-positive children: two mothers were positive for HBeAg and another two were negative for HBeAg. This finding is worrisome and could be due to several potential reasons.

First, HBV birth-dose vaccine was not provided on time. Of four children infected despite having been born to low-risk mothers, three received the HBV birth-dose vaccine later than 24 h after birth. Second, these children were assessed for HBsAg much later than the recommended period for mother-to-child transmission assessment (7–12 months),[3] increasing the possibility of intrafamilial horizontal transmission, which is particularly common from infectious older siblings in Africa.[26] However, this hypothesis is unlikely to have

Maternal characteristics					Child characteristics						Homology of whole HBV genome in mother-child pairs (%)
Age (years)	HBeAg	HBcrAg (log$_{10}$ U/mL)	HBV DNA (log$_{10}$ IU/mL)	HBV genotype (Sanger)	Sex	Age at follow-up (years)	Birth order	Timing of HBV birth-dose vaccine (h)	HBV DNA using dried blood spot (log$_{10}$ IU/mL)	HBV genotype (Sanger)	
HBsAg-positive children (n=39)											
1	18	Positive	3·6	8·8	E	Female	5·5	1st	<24	6·4	E	..*
2	30	Positive	8·1	8·9	E	Male	2·7	1st	24–48	7·4	E	..†
3	19	Positive	8·0	8·6	E	Male	4·8	1st	24–48	2·6	..	..
4	19	Positive	8·1	8·6	E	Male	3·0	1st	<24	7·3	E	100%
5	21	Positive	8·2	8·5	E	Female	1·9	1st	<24	6·1	E	100%
6	18	Positive	8·0	8·5	E	Female	2·7	1st	<24	6·2	E	..
7	30	Positive	8·0	8·8	E	Female	3·3	1st	<24	7·7	E	100%
8	19	Positive	8·1	8·3	E	Female	4·7	1st	<24	7·0	E	100%‡
9	18	Positive	7·3	7·3	E	Male	3·1	1st	48–96	6·6	E	99·97%
10	18	Positive	7·8	8·4	D	Male	5·9	1st	<24	7·5	D	100%
11	29	Positive	7·9	8·8	E	Female	1·7	1st	<24	7·4	E	100%
12	20	Positive	7·9	8·6	E	Female	4·7	1st	<24	7·5	E	99·97%
13	20	Positive	8·1	8·8	E	Male	2·0	1st	<24	7·5	E	100%
14	36	Positive	8·3	8·7	E	Male	3·5	1st	<24	7·0	E	100%
15	27	Positive	8·4	8·7	..	Female	4·4	1st	24–48	7·0	..	..
16	30	Negative	4·8	3·2	E	Female	3·1	1st	<24	5·7	E	..
17	31	Positive	7·8	8·1	E	Male	5·8	1st	<24	7·6	E	100%
18	33	Positive	7·8	7·7	E	Male	4·0	2nd	24–48	7·6	E	100%
19	16	Positive	7·8	8·8	..	Female	2·3	1st	<24	7·0	..	..
20	19	Positive	5·0	2·8	E	Male	4·8	1st	24–48	Undetectable	E	..
21	20	Negative	3·6	0·7	..	Female	3·2	1st	48–96	Undetectable	E	..
22	22	Positive	8·1	8·3	E	Male	3·5	1st	<24	5·4	..	..
23	23	Positive	8·4	3·0	E	Female	2·0	2nd	48–96	6·5	E	100%
24	17	Positive	6·6	8·7	E	Female	5·8	1st	<24	6·8	E	99·97%
25	22	Positive	7·8	8·1	E	Male	3·2	1st	24–48	6·3	E	99·97%
26	30	Positive	7·6	8·2	E	Male	1·7	1st	24–48	7·8	E	100%
27	24	Positive	8·2	8·6	E	Male	1·3	2nd	<24	7·5	..	99·34%
28	30	Positive	7·9	9·1	E	Male	6·6	1st	<24	Undetectable	..	..
29	19	Positive	7·9	6·8	E	Female	5·7	1st	48–96	Undetectable	..	..
30	NA	Positive	7·8	8·4	E	Male	5·8	1st	<24	6·4	E	100%
31	32	Positive	8·0	8·6	E	Male	1·0	1st	<24	6·7	E	100%
32	28	Positive	8·2	8·4	E	Female	6·7	1st	24–48	6·3	E	99·97%
33	20	Positive	8·1	8·3	E	Male	6·9	1st	<24	6·2	E	99·97%
34	30	Positive	7·4	8·6	E	Female	8·0	1st	<24	Undetectable	..	..
35	21	Positive	8·0	8·7	E	Male	4·2	1st	<24	4·0	E	100%
36	19	Positive	8·0	8·5	E	Female	1·0	1st	24–48	4·9	E	100%
37	26	Positive	8·0	8·7	E	Female	0·9	1st	24–48	5·0	E	100%
38	20	Positive	8·1	8·5	E	Male	4·4	1st	24–48	4·5	E	100%
39	19	Positive	7·4	8·2	E	Female	1·7	2nd	<24	4·3	E	100%
HBsAg-negative children with detectable HBV DNA (n=2)§											
40	16	Positive	7·8	8·1	E	Male	6·3	1st	<24	2·5	..	..
41	20	Positive	8·1	8·9	E	Male	3·2	1st	24–48	4·6	..	..

HBsAg=HBV surface antigen. HBV=hepatitis B virus. HBeAg=HBV e antigen. HBcrAg=HBV core-related antigen. NA=not available. *MinION HBV sequence was obtained only for the child. †MinION HBV sequence was obtained only for the mother. ‡HBsAg escape mutation was found in the mother-child pair with a substitution at codon 129 (Gln129His). §Plasma samples were obtained from both children, and negative HBsAg and positive HBV core antibody were further confirmed by chemiluminescent microparticle immunoassay.

Table 4: Characteristics of mother-child pairs in HBsAg-positive children (n=39) and HBsAg-negative children with detectable HBV DNA (n=2)

occurred because the risk of horizontal transmission is minimal in children who completed three doses of HBV vaccine.[16] Moreover, three of these children were firstborns, and the child with a second birth order had whole-genome sequencing showing 100% homology to her mother. Third, maternal viral load was determined during the second trimester; this concentration might have been different from the concentration at the time of delivery. Fourth, a cold chain might have been disrupted. Although we have closely monitored storing temperatures at Tokombéré District Hospital, no detailed record was available during the transport. In HBsAg-negative children, a decrease in the prevalence of positive anti-HBs was documented with an increase in their age at assessment. This finding should not, however, indicate the absence of immunogenicity of these vaccines; it is well known that in vaccinated individuals, anti-HBs wanes rapidly over time, but the majority preserve vaccine-induced immunological memory that can be verified by an anamnestic response to a challenge dose of HBV vaccine.[27] Fifth, the vaccines used in this study might be less effective in preventing HBV genotypes circulating in this area. The efficacy of Engerix-B, originally developed from HBV genotype A2, against non-A2 subgenotypes has been questioned.[28] All four children born to low-risk mothers were infected with genotype E, and all genotype E strains in this study had a mutation leading to an aminoacid substitution at position 140 in the "a" determinant of HBsAg. The Thr140Ser substitution, frequently observed in genotype E, might potentially evade neutralising antibodies induced by vaccination.[29] Sixth, the presence of escape mutants might be responsible for the vaccine failure. In our study, however, the escape mutation Gln129His was found only in one mother-child pair, suggesting a limited role for this mutation in our settings.

We identified several risk factors for child HBsAg positivity. Younger HBV-infected mothers had a higher risk of child HBsAg positivity than did older mothers, possibly because HBeAg prevalence decreased with an increase in maternal age. These findings support the use of maternal age as a proxy for mother-to-child transmission risk in an epidemiological study.[30] HBcrAg is known to be closely correlated with serum HBV DNA concentrations in treatment-naive HBV-infected individuals,[22] but its performance in predicting the mother-to-child transmission risk has never been assessed. The sensitivity and specificity of HBcrAg were comparable to those of HBV DNA, suggesting that this might be a useful alternative to nucleic acid testing to identify high-risk pregnant women.

The study's strength was the use of ONT MinION long-read sequencing technology, which confirmed a very high rate of identity in HBV strains in infected mother-child pairs. This finding strongly suggested that the mothers were the source of HBV transmission. Although this finding might not preclude the possibility of horizontal transmission from an older sibling who was vertically infected by their mother, the majority of HBsAg-positive children were firstborns. Our study also has limitations. Continuum of care was disrupted largely because of social and political instability caused by a terrorist organisation (Boko Haram). The supply of HBsAg screening tests was interrupted intermittently; a curfew prohibited women from delivering at health facilities during the night; and many of the families were forced to displace internally leading to the high rate of children lost to follow-up. However, our previous virological assessment of 1276 pregnant women, who had been consecutively screened for HBV between January, 2009, and April, 2010, at the time of antenatal care in Tokombéré, showed a similar distribution of mother-to-child transmission risk factors to the current analysis: the proportion of HBeAg-positive women was 22·7% in the previous study versus 21·4% in the current study and the proportion with high viral loads of 5·3 $\log_{10}$ IU/mL or more was 23·6% in the previous study versus 20·4% in the current study.[14] These findings suggest that the risk of selection bias due to the high rate of children lost to follow-up in the current study might be low. Our study also has limited external validity because it was done in a rural district of north Cameroon known to have high HBV prevalence[30] and political unrest.

In conclusion, our study documented an important residual risk of mother-to-child transmission, confirmed by whole-genome sequencing, despite the timely administration of the HBV birth-dose vaccine. This finding clearly indicates an important unmet need for an additional strategy to prevent mother-to-child transmission in high-prevalence regions of Africa, and underlines the importance of implementing the new WHO recommendation of peripartum antiviral therapy in high-risk pregnant women, in addition to the infant immunoprophylaxis including the timely HBV birth-dose vaccine.

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