厚生労働科学研究費補助金（肝炎等克服政策研究事業）
令和４年度　研究報告書
全国規模の肝炎ウイルス感染状況の把握及びウイルス性肝炎 elimination に向けた
方策の確立に資する疫学研究

ブルキナファソにおける 1622 例の妊婦を対象とした HBV 治療と母子感染防止効果の把握のための前向き血清疫学研究（中間報告）

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研究要旨

B 型肝炎ウイルス（HBV）は 9 種類の Genotype（A~J 型）に分類されており、Genotype には地域特異性や臨床経過の違いがあることが知られている。日本では HBV Genotype C、B の順に多いが、近年海外から持ち込まれたと考えられる Genotype A 型も増加傾向にある[1]。グローバル化に伴って、現在の日本では一般的ではない HBV Genotype についても、今後持ち込まれる可能性がある。

西アフリカに位置するブルキナファソは、HBV 感染の高浸淫国であり、2006 年からすべての乳児に対し出生後 2, 3, 4 ヶ月の HB ワクチン接種が開始しているが、2018 年に実施した母子 240 組を対象とした調査では HBs 抗原陽性率は母親の 6.3%、小児 0.8%であった[2]。主要な Genotype は我が国とは異なる E と A である[2]。WHO の HBV 母子感染防止ガイドラインでは出生時 HB ワクチン投与および HBIG 投与等の予防対策に加え、HBV DNA 200,000 IU/mL または HBe 抗原陽性の妊婦に対する抗ウイルス療法を推奨している。ブルキナファソの同ガイドラインでも同様に HBV ウイルス量の高い妊婦への抗ウイルス療法（出産前後の 3 ヶ月計 6 か月）を推奨しているが、治療適応例の診断精度の問題や保険診療での負担ではない等課題があり広く実施されていない。

本研究は、同国農村部の大規模妊婦集団を対象とし、HBs 抗原陽性率と HBV 母子感染の高リスク妊婦（高ウイルス量）の割合、治療適用となるウイルス量（HBV DNA>200,000 IU/mL）の代替指標としての HBe 抗原の有用性、HBs 抗原陽性妊婦への抗ウイルス療法の効果および HBV 母子感染の実態評価を目的とした、縦断的血清疫学調査研究である。

本研究は、ブルキナファソ国立保健科学研究所中央西部地区ナノロ臨床研究ユニットとの国際共同研究（P.I.田中純子）であり、広島大学疫学倫理審査委員会およびブルキナファソ国立保健科学研究所倫理審査委員会の承認を得て実施した (approval number: E-2137, 2020-8-145)

2021 年 2 月から 11 月の期間に、ヤコ地域の 3 医療機関で妊婦健診を受けた全妊婦のうち同意を得た 1,622 人（平均年齢 25.1±6.0 歳）を対象に、HBs 抗原迅速診断検査を実施し、陽性の場合には乾燥濾紙法(DBS)を用いた血液検体採取を行った。DBS は液検体の保管と感度が優れている HemaSpot を採用した。DBS 検体は日本に冷蔵輸送し、化学発光酵素免疫測定法（CLEIA）による HBs 抗原（再確認）、HBe 抗原、HBe 抗

体測定、real-time PCR、nested PCR による HBV DNA ウイルス量の測定、Genotype の決定を行った。【Study1】。

HBs 抗原陽性の妊婦は追跡調査対象者に登録され、出産 3 ヶ月前から出産後 3 ヶ月まで 6 か月間テノホビルの予防投与を受けた。生まれた児は、ブルキナファソのガイドラインに従って出生時とその後 4 回（1、2、3、4 ヶ月）の Hep B ワクチン接種を受けた。生後 6 ヶ月後に児の HBs 抗原検査を行い、母子感染予防策の成果を評価する予定である【Study2】。

現在までに【Study1】を完遂し、以下の結果を得た。

1. 妊婦 1,622 人のうち、HBs 抗原迅速検査陽性は 106 人（6.5%、平均年齢 25.9±6.0 歳）であった。DBS 検体の分析は 106 検体中 102 検体で実施可能であった。HBsAg 陽性例のうち HBe 抗原陽性 22.6%、HBe 抗体陽性 66.7%であり年齢が高いほど HBe 抗原陽性率は低く、HBe 抗体陽性率は高い傾向がみられた。HBV ウイルス量の分析は 94 例で可能であり、HBe 抗原陽性例におけるウイルス量は HBe 抗原陰性例よりも有意に高値（各中央値：193,580.0 IU/mL、12,011 IU/mL）であったが、HBV DNA 量が 200,000 IU/mL 以上の例においても HBe 抗原が陰性を示す例が複数認められた。ウイルス量高値群（HBV DNA>200,000 IU/mL）は HBV 陽性妊婦の 19.1%を占めた。
2. HBV 遺伝子配列の SP 領域と S 領域の nested PCR により Genotype の判定は 63 例で可能であり、主要な Genotype は E（58.7%）と A（36.5%）であった。Genotype E 例におけるウイルス量は、Genotype A 例よりも有意に高値（各中央値：76,233 IU/mL、23,201 IU/mL）であり、ウイルス量高値群（HBV DNA>200,000 IU/mL）の割合は、Genotype E 例において Genotype A 例よりも有意に高かった（35.1% vs 8.7%）。
3. WHO が定めた抗ウイルス剤投与のカットオフ値である 200,000 IU/mL 以上のウイルス量を判定するために、HBeAg 測定系を代替で用いた場合の精度については、Genotype E では、感度 53.8%、特異度 91.7%、一方 Genotype A では感度 100%、特異度 66.7%であった。.

以上より、

妊婦 1,622 人を対象としたブルキナファソとの国際共同研究により、ブルキナファソでは妊婦の HBs 抗原陽性率が依然として高いこと、また、HBs 抗原陽性妊婦の約 5 分の 1 は HBV 母子感染のリスクが高いことを同国で初めて提示し、2030 年までに設定されたウイルス肝炎撲滅への道筋として、早急に妊婦の HBV スクリーニングの導入が必要であることを示唆した。さらに、HBV Genotype E が主要感染株であるブルキナファソを含む地域等においては、HBV 母子感染防止のための治療対象とする HBV 陽性妊婦の基準に HBV DNA 量測定の代替として HBe 抗原測定系を用いた場合には、治療対象とすべきウイルス量の多い妊婦を除外する可能性があることを明らかにした。

現在、【Study2】が進行中であり、HBV 陽性妊婦への抗ウイルス療法の効果、出生児のフォローアップすなわち HBV 母子感染の実態の評価を行っている。

1. 国立感染症研究所. https://www.niid.go.jp/niid/ja/kansennohanashi/321-hepatitis-b-intro.html
2. Lingani M, Akita T, Tanaka J et al. The Changing Epidemiology of Hepatitis B and C Infections in Nanoro, Rural Burkina Faso : A Random Sampling Survey. BMC Infect Dis. 2018; 20:46.

A. 研究目的

Japan has a successful history of combating Hepatitis B virus (HBV) with measures to eliminate mother-to-child transmission (MTCT) implemented since 1986. This project involved testing all pregnant women for hepatitis B surface antigen (HBsAg) and administering hepatitis B immunoglobulin (HBIG) and vaccines to infants born to HBsAg-positive mothers. As a result, MTCT of HBV in Japan has significantly decreased,[1]

making it a global leader in HBV control. The main HBV genotypes in Japan are genotypes B and C and countermeasures have been established to target these genotypes. However, as the foreign community in Japan increases, the possibility of importing other HBV genotypes not commonly found in Japan is a concern. It is essential to understand the characteristics of these genotypes.

Despite vaccination and HBIG, MTCT can still occur if maternal viral load is >200,000 IU/mL, prompting the World Health Organization (WHO) to recommend antiviral treatment in pregnant women with viral load>200,000 IU/mL.[2] In countries where viral load is not available, HBeAg is recommended as a replacement. Japan has not implemented this recommendation due to concerns about the safety of antivirals during pregnancy.

Burkina Faso in West Africa has a high prevalence of HBV, with predominance of genotypes E and A.[3] In a preliminary study in Nanoro, rural Burkina Faso in 2018 (PI: Prof. J. Tanaka), HBV prevalence in mothers of children less than 5 years was 6.3%, indicating a risk for MTCT.[4] Although Burkina Faso included antiviral prophylaxis in their MTCT prevention guidelines, the guidelines are not widely implemented because there is no national health insurance, so data about the success rate of this prevention strategy in Burkina Faso is lacking.

The purpose of this study was to confirm the prevalence of HBsAg among pregnant women in rural Burkina Faso, determine the proportion of those at high risk of MTCT, assess the performance of HBeAg as alternative to viral load testing and assess the success rate of antiviral prophylaxis in preventing MTCT in Burkina Faso.

B．研究方法

This research was conducted in collaboration with the Clinical Research Unit of Nanoro, Center-West Regional Direction of the National Institute for Health Science Research, Burkina Faso.

1. Study design and methods

This is a longitudinal study conducted in 3 health centers in rural Burkina Faso. Between February and November 2021, 1,622 pregnant women visiting the 3 health centers for antenatal care were registered and tested for HBsAg by a WHO-approved rapid diagnostic test (Determine HBsAg 2, Abbott, Japan) and blood samples were collected by DBS (Hemaspot, Spot on Science, USA) among those HBsAg positive.

HBsAg-positive pregnant women were enrolled in a follow-up study and received tenofovir prophylaxis from 3 months before delivery to 3 months after delivery, and their infants received HB vaccine at birth followed by 4 doses (1,2,3,4 months) as per Burkina Faso guidelines. DBS samples were collected at delivery and 6 months in mothers to evaluate the decrease in maternal viral load and at 6 months in babies to evaluate their HBsAg status.

Pregnant women registration is now complete and 105 DBS samples from HBsAg-positive were collected and shipped to Japan for laboratory analyses. The follow-up study is ongoing (Figure 1).

2. Laboratory analysis method

Total 105 DBS samples from HBsAg-positive pregnant women at registration were collected and shipped to Japan for serological and molecular analyses.

For serological analyses, 3 fins of DBS were extracted and eluted with 600uL of elution buffer, before analyses by chemiluminescent enzyme immunoassay (CLEIA). First, HBsAg was tested to confirm HBV infection, then HBeAg and HBeAb were tested in HBsAg-positive cases.

For molecular analyses, 1 DBS fin was used to extract HBV DNA and perform real-time PCR to quantify HBV viral load, and nested PCR followed by partial genome sequencing to identify HBV genotypes (Figure 2).

The following materials were used for laboratory analyses:

1) HBV seromarkers

Reagents

HBsAg: Lumipulse® HBsAg-HQ (COI=0.005, Se=89.3%, Sp=100%)[5]

HBeAg: Lumipulse® HBeAg (COI=0.2, Se=100%, Sp=100%)[5]

HBeAb: Lumipulse® HBeAb (COI=37.9, Se=100%, Sp=100%)[5]

Machine : Lumipulse G1200 (Fujirebio, Tokyo, Japan)

2) Real time PCR

Reagent: TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific, Tokyo, Japan)

Machine: Applied Biosystems StepOne (Thermo Fisher Scientific, Tokyo, Japan)

3) Nested PCR

Reagent: PrimeScript One-Step RT-PCR Kit Ver.2 (Takara Bio, Shiga, Japan)

Machine: MiniAmp Plus Thermal Cycler (Thermo Fisher Scientific, Tokyo, Japan)

4) Partial Genome sequencing by sanger method

Reagent: BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Tokyo, Japan)

Machine: SeqStudio Sequence Analyzer (Thermo Fisher Scientific, Tokyo, Japan)

5) Phylogenetic tree and HBV genotype identification:

Method: Neighbor-joining

Software: MEGA 10

3. Data analysis

HBsAg prevalence was calculated among pregnant women who tested positive for HBsAg by rapid test.

The prevalence of HBeAg and HBeAb was calculated among HBsAg-positive in DBS.

Factors associated with HBsAg positivity among pregnant women were analyzed by multivariable logistic regression. The level of significance of all statistical analyses was 0.05.

4. Ethical considerations

The study received ethical approval from the Burkina Faso National Ethics Committee (approval number 2020-8-145) and the Ethics Committee for epidemiological research of Hiroshima University (approval number E-2137).

C. 研究結果

1. Prevalence and factors associated with HBsAg-positive in pregnant women

A total of 1,622 pregnant women were registered, of whom 106 tested positive for HBsAg by rapid test, giving a prevalence of 6.5% (Figure 3). By multivariable logistic regression, the factors associated with HBsAg positive were 25-34 years (aOR=2.24, 95%CI, 1.28-3.93, p=0.005), never heard of HBV (aOR=1.63, 95%CI, 1.07-2.49, p=0.022), and Female genital mutilation (aOR=2.25, 95%CI, 1.19-4.24, p=0.0139) (Table 1).

2. Prevalence of HBeAg and HBeAb

Of the 106 pregnant women HBsAg positive by rapid test, one participant did not provide DBS sample, and 3 were negative for HBsAg by CLEIA. Among the remaining 102 samples, HBeAg prevalence was 22.6% (23/102) and decreased with increasing age (p=0.040) (Figure 3). On the other hand, HBeAb was positive in 66.7% (68/102) and increased with age (p=0.005).

3. HBV viral load distribution

Eight samples did not have enough DBS fins for real-time PCR, so HBV DNA was quantified in 94 samples.

Median viral load was 18,317 IU/mL and was significantly higher in HBeAg-positive (193,580 IU/mL) than HBeAg-negative pregnant women (12,011 IU/mL, p<0.001) (Figure 4).

The proportion of pregnant women with viral load ≥200,000 IU/mL was 19.1% (18/94).

4. HBV genotypes distribution

HBV partial sequencing was successfully performed in 63 samples and genotype E was predominant at 58.7% (37/63), followed by genotype A (36.5%, 23/63), B (3.2%, 2/63) and C (1.6%, 1/63) (Figure 5).

5. HBeAg Status and Viral Load by HBV Genotypes

The mean age of pregnant women was not significantly different between genotype A (25.4 ± 5.8 years) and genotype E (25.1 ± 5.1 years) (p=0.352). HBeAg positivity was higher in genotype A (36.1%, 9/23) than in genotype E (24.3%, 9/37). On the other hand, median viral load was higher in genotype E (23,201 IU/mL) compared to genotype A (76,233 IU/mL). The rate of pregnant women with viral load ≥200,000 IU/mL was also higher in genotype E (35.1%, 13/37) than in genotype A (8.7%, 2/23) (Figure 6).

6. Performance of HBeAg to identify HBV DNA≥200,000 IU/mL

Among 94 samples with viral load results, the sensitivity of HBeAg to predict viral load>200,00 IU/mL was 55.6% (10/18) and the specificity was 86.8% (66/76), meaning that some pregnant women had high viral load but were HBeAg-negative.

The sensitivity was lower in genotype E (53.8%, 7/13) than in genotype A (100%, 2/2), while the specificity was higher in genotype E (91.7%, 22/24) than in genotype A (66.7%, 14/21) (Table 2).

D. 考察

1. This study showed that HBV genotype E has a higher viral load than genotype A, meaning that genotype E is more transmissible than genotype A. If HBV genotype E enters and spreads in Japan, it might have a greater impact on the transmission of HBV than genotype A.
2. It was also found that in genotype E, HBeAg does not predict well viral load>200,000 IU/mL, the threshold for which WHO recommends antiviral prophylaxis. Some pregnant women with high viral load were HBeAg-negative in genotype E, giving a sensitivity of 53.8% in this genotype. In another study that our group conducted in Cambodia, the sensitivity was 92.3% in genotype C and 100% in genotype B,[6] which are also the main genotypes in Japan (Table 2). This finding suggests that viral load should be preferred over HBeAg when assessing MTCT risk in genotype E.
3. The follow-up study among infants is now ongoing. The results will help to have a better insight on MTCT prevention using antiviral treatment during pregnancy and provide data for Japan to consider the implementation of such strategy in HBV-infected pregnant women of African descent living in Japan.

E. 結論

This international collaborative research with Burkina Faso has provided a better understanding of HBV genotypes that are not common in Japan but could potentially be introduced due to globalization. Genotype E was found to have higher viral load than genotype A and could change the epidemiological situation of Japan if it enters the country.

Genotype E was found to have a poor sensitivity to predict the WHO cutoff for antiviral prophylaxis (200,000 IU/mL). Genotype E is mainly found in West Africa, where viral load testing is not always available, so it is urgently needed to develop affordable and easy-to-use point-of-care viral load tests.

This collaborative study enabled Japan to

participate in the global fight to eliminate HBV by supporting Burkina Faso and providing Japan's experience and expertise in HBV control.

F. 健康危険情報

特になし。

G. 研究発表

1. 論文発表

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2. 学会発表

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H. 知的財産権の出願・登録状況（予定を含む。）

特になし。

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1. Sugiyama A, Yamashita M, Tanaka J et al. Epidemiological assessment of interventions to eliminate mother-to-child transmission of hepatitis B virus in Japan. GastroHep. 2021;3:72–79
2. World Health Organization. Prevention of Mother-to-Child Transmission of Hepatitis B Virus: Guidelines on Antiviral Prophylaxis in Pregnancy. 2020;(July):1-58.
3. Lingani M, Akita T, Tanaka J et al. High prevalence of hepatitis B infections in Burkina Faso (1996–2017): a systematic review with meta-analysis of epidemiological studies. BMC Public Health. 2018;18(1):551.
4. Lingani M, Akita T, Tanaka J et al. The Changing Epidemiology of Hepatitis B and C Infections in Nanoro, Rural Burkina Faso : A Random Sampling Survey. BMC Infect Dis. 2018; 20:46.
5. E B, Ko K, Tanaka J et al. Dried blood spot-based detection of serological profiles of hepatitis B and C infections and their prevalence in Cambodia. GastroHep. 2021;3(4):247-253.
6. EB, Ko K, Tanaka J et al. Residual risk of mother-to-child transmission of HBV despite timely Hepatitis B vaccination: a major challenge to eliminate hepatitis B infection in Cambodia. BMC Infectious Diseases. 2023; 23:261

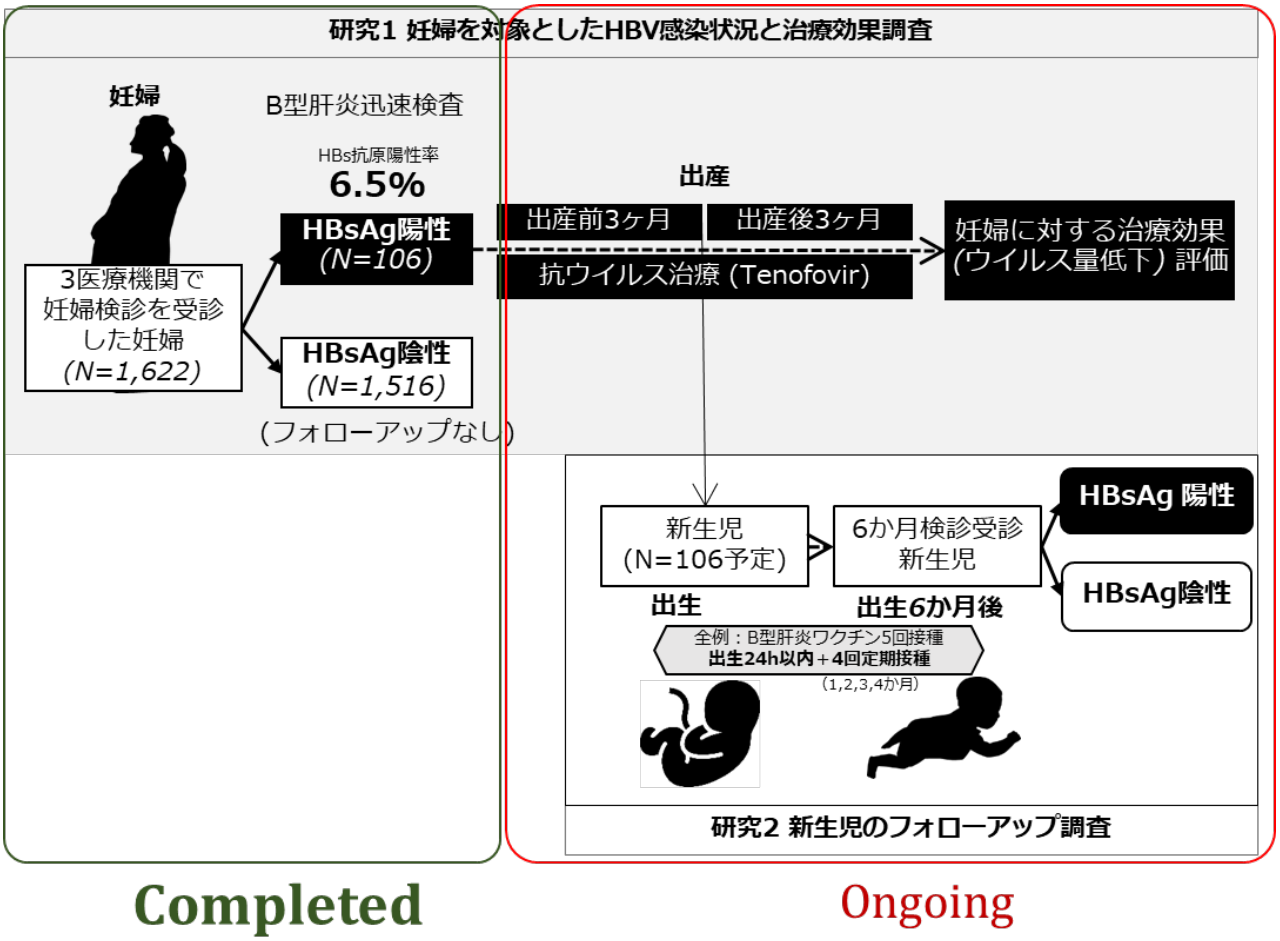

Figure 1. Study protocol

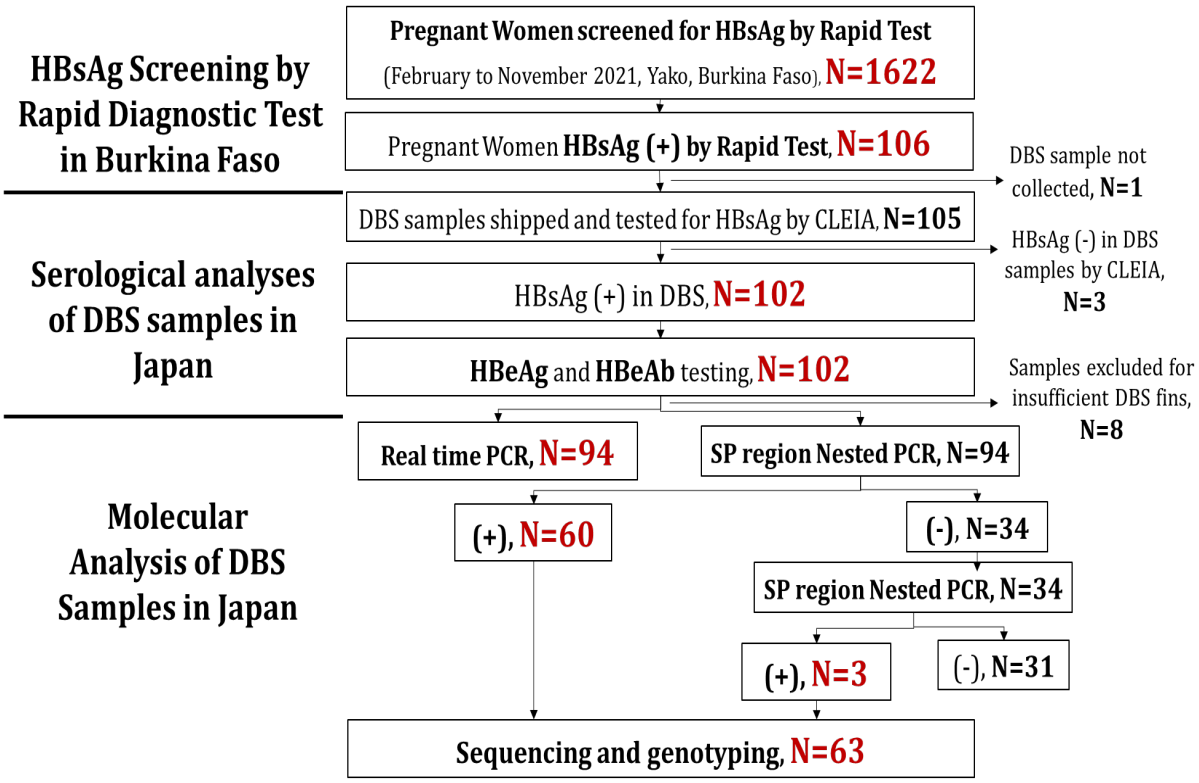

Figure 2. Flowchart of the study in pregnant women at registration

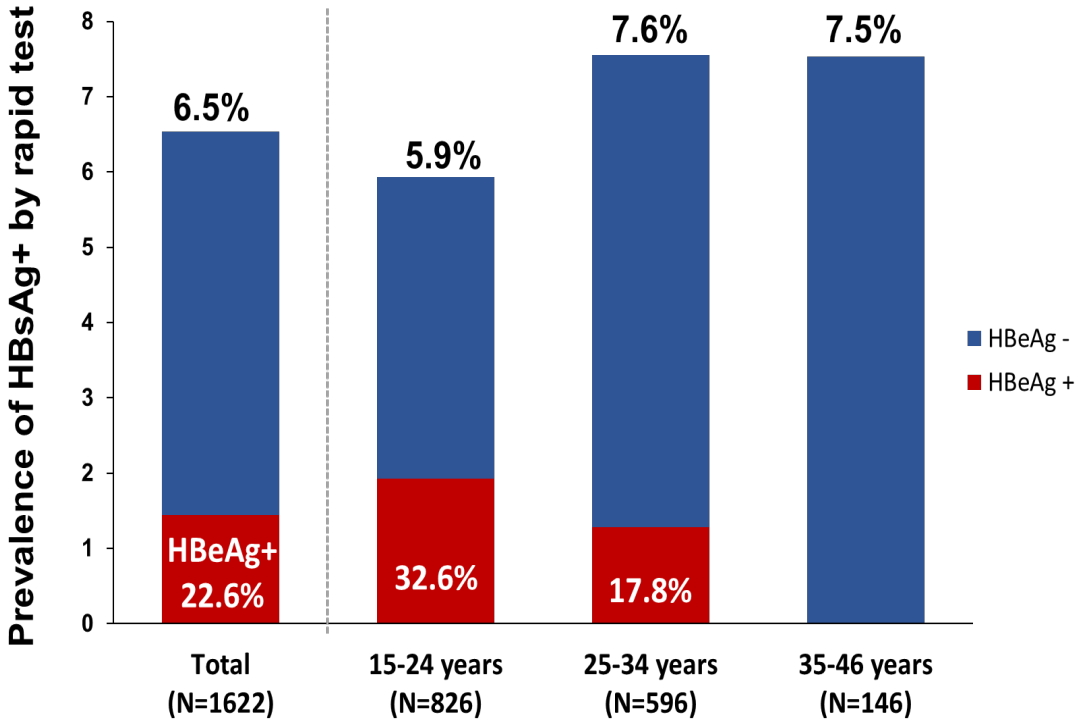

Figure 3. HBsAg prevalence among 1622 pregnant women by rapid test and HBeAg prevalence among 102 HBsAg positive pregnant women in rural Burkina Faso

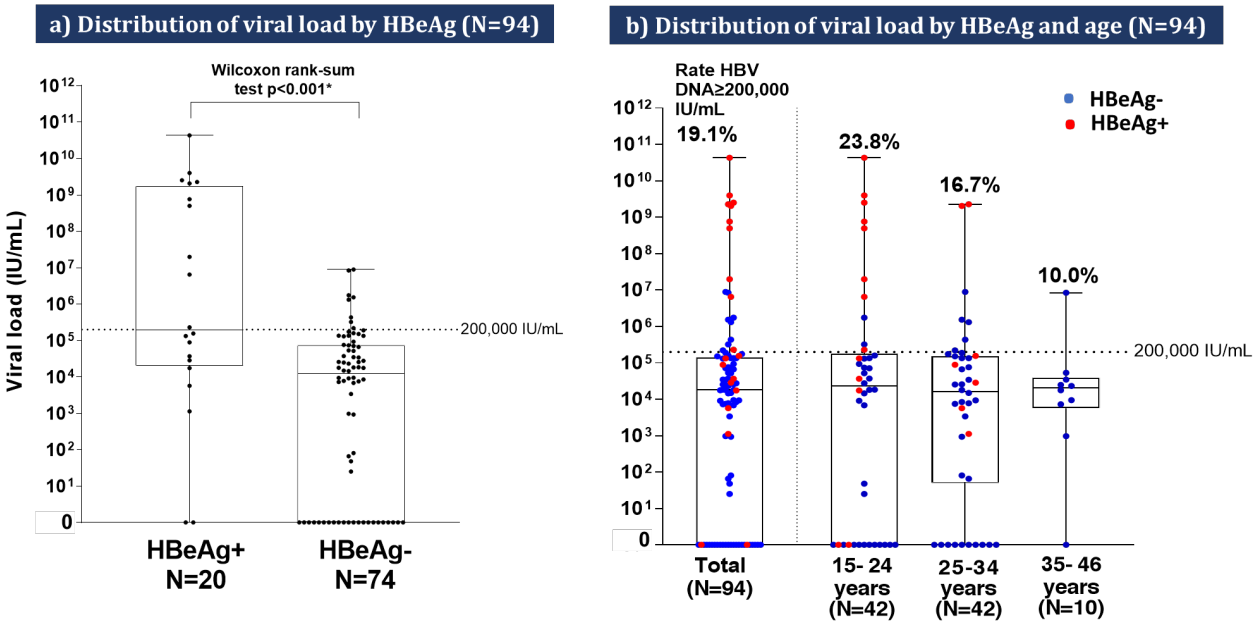

Figure 4. Distribution of HBV DNA by a) HBeAg and b) HBeAg and age among pregnant women in rural Burkina Faso. The red dots show HBeAg-positive cases, and the blue dots represent HBeAg-negative cases. Numbers on the top of each graph represent the percentage of pregnant women with HBV DNA ≥ 200,000 IU/ mL, the recommended cut-off for antiviral prophylaxis.

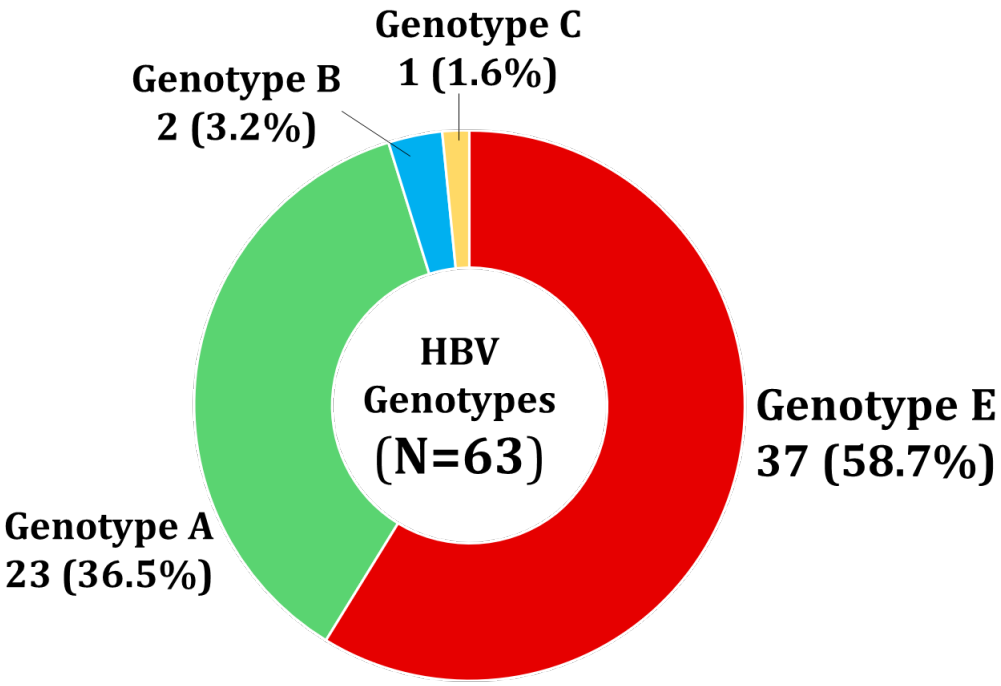

Figure 5. Distribution of HBV genotypes among 63 pregnant women in Yako, rural Burkina Faso

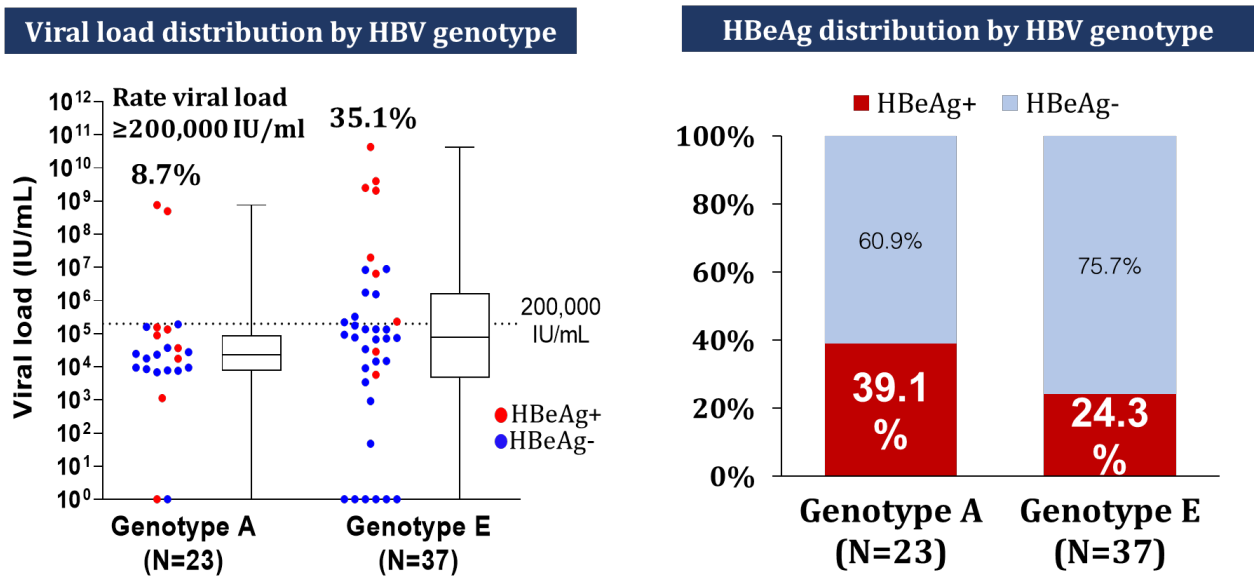

Figure 6. Distribution of a) HBV DNA by genotype and b) HBeAg by genotype among pregnant women in rural Burkina Faso. The red dots show HBeAg-positive cases, and the blue dots represent HBeAg-negative cases. Numbers on the top of each graph represent the percentage of pregnant women with HBV DNA ≥ 200,000 IU/ mL, the recommended cut-off for antiviral prophylaxis.

Table 1. Factors associated with HBsAg positive among pregnant women in Yako, rural Burkina Faso

Variable	Category	Univariable logistic regression		Multivariable logistic regression	
		OR (95% CI)	p-value	aOR (95% CI)	p-value
Age group (years)	15-24	1		1	
	25-34	1.30 (0.85-1.97)	0.227	2.24 (1.28-3.93)	0.005
	35-46	1.29 (0.66-2.55)	0.46	2.15 (0.89-5.18)	0.089
Parity	No previous delivery	1		1	
	1 previous delivery	1.02 (0.6-1.73)	0.938	0.46 (0.23-0.91)	0.509
	>1 previous delivery	0.87 (0.53-1.42)	0.574	0.83 (0.47-1.45)	0.025
Ever heard of HBV	Yes	1		1	
	No	1.74 (1.17-2.6)	0.007	1.63 (1.07-2.49)	0.022
Transfusion	Yes	1.25 (0.29-5.36)	0.768	1.43 (0.31-6.52)	0.646
	No	1		1	
Surgery	Yes	0.93 (0.28-3.04)	0.903	0.57 (0.13-2.49)	0.456
	No	1		1	
Piercing	Yes	1.33 (0.77-2.31)	0.302	0.79 (0.42-1.96)	0.462
	No	1		1	
Scarification	Yes	1.52 (1.01-2.28)	0.043	1.26 (0.80-1.96)	0.319
	No	1		1	
Female Genital Mutilation	Yes	1.89 (1.11-3.22)	0.019	2.25 (1.19-4.24)	0.013
	No	1		1	

N=1,453; R^2 = 0.033; Model p=0.008.

Table 2. Performance of HBeAg to detect HBV DNA ≥ 200,000 IU/mL

	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)
Burkina Faso		
Total samples (N=94)	55.6 (30.8-78.5)	86.8 (77.1-93.5)
Genotype A (N=23)	100 (15.8-100)	66.7 (43.0-85.4)
Genotype E (N=37)	53.8 (25.1-80.8)	91.7 (73.0-99.0)
Cambodia[6]		
Total samples (N=67)	94.7 (74.0-99.9)	79.2 (65.0-98.5)
Genotype B (N=19)	100 (54.1-100)	92.3 (64.0-99.8)
Genotype C (N=42)	92.3 (64.0-99.8)	75.9 (56.5-89.7)
WHO[2]	88.2 (83.9–91.5)	92.6 (90.0–94.5)