

# Prevention of mother-to-child transmission of hepatitis B virus in Burkina Faso: Screening, vaccination and evaluation of post-vaccination antibodies against hepatitis B surface antigen in newborns

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## Abstract

The low rate of screening for hepatitis B virus (HBV) in pregnant women is a high-risk factor for its vertical transmission. The objectives of this study were: i) to screen pregnant women for HBV infection; ii) vaccinate all children from birth against HBV regardless their mother HBV status; and iii) evaluate after 7 months of birth the level of their AbHBs among babies who received HBV vaccine at birth. Serological markers of HBV (HBsAg, HBeAg, AbHBs, AbHBe, and AbHBc) were determined on venous blood samples from 237 pregnant women and their children using the Abon Biopharm Kit. One hundred and two (102) children received the three doses of the EUVAX B® vaccine respectively at birth, two months and four months of life. Seven months after

delivery, venous blood samples were collected from mothers and their children. Antibodies against hepatitis B surface antigen (AbHBs) were measured in vaccinated children using the ELISA Kit *AbHBs Quantitative EIA*. DNA extraction was performed on samples from HBV-seropositive mothers and their children using the *Ribo Virus (HBV Real-TM Qual)* Kit and for Real Time PCR, the *HBV Real-TM Qual* Kit was used. Serological diagnosis in pregnant women revealed 22 (9.28%) hepatitis B surface antigen (HBsAg) positive samples of which 21 were positive for viral DNA by real-time PCR. Among the 22 HBsAg+ women, five (05) transmitted the virus to their children with a vertical transmission rate of 22.73%. A transmission rate of 23.81% (5/21) was found with the PCR method. Analysis of AbHBs levels revealed that 98.31% of the children had an average concentration of  $218.07 \pm 74.66$  IU/L, which is well above the minimum threshold for protection (11 IU/L). This study has confirmed that vertical transmission of HBV is a reality in Burkina Faso and that vaccination at birth would significantly reduce this transmission.

## Introduction

Currently, the World Health Organization (WHO) estimates that 257 million people live with hepatitis B virus (HBV) infection worldwide.<sup>1</sup> HBV is responsible for more than 686,000 deaths per year and this infection is considered as the second most common cause of cancer deaths after tobacco.<sup>1</sup> Africa is the most affected region of the world with approximately 150 million cases of chronic hepatitis B and C associated with the risk of developing cirrhosis or liver cancer.<sup>2</sup> Burkina Faso (BF) which is a HBV high endemic area according to WHO classification has prevalence well above 8%.<sup>3-6</sup>

A study carried out at the University Hospital Yalgado Ouedraogo of Ouagadougou (CHU-YO) showed a prevalence of 4.1% in newborns to HBV infected mothers with a vertical transmission rate of 37.1%.<sup>7</sup> Another study conducted in 2002 at the Saint Camille Hospital of Ouagadougou found a vertical transmission rate of 25.0%.<sup>8</sup> Thus, according to McMahon *et al.*<sup>9</sup> vertical transmission remains the most important mechanism of HBV chronic infections in Africa. The age at first exposure to HBV plays a very important role in the evolution of infection. Children born to mothers with active hepatitis B, who are positive for HBV “e” antigen (HBeAg+), are at high risk of infection at birth or dur-

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ing early childhood and of becoming chronic carriers.<sup>10</sup> Moreover, the infection could occur during childhood and would imply transmission though breastfeeding. In addition, since the hepatitis B virus can be transmitted by blood, saliva and other fluids of the infected human organism, infected parents could transmit the virus to their infants and children as they take care of them including some traditional methods of caring for insert bites by applying saliva to site sites. If the latter transmission route plays a key role in HBV infection, then the local density of these blood-sucking arthropods would be a relevant factor to take into account during awareness against hepatitis B infection.<sup>3</sup>

Following this infection, approximately 25% of these children are at greater risk of death because they may develop hepatocellular carcinoma or cirrhosis in adulthood.<sup>9</sup> The low rate of screening for HBV in preg-

nant women is therefore a high risk factor for vertical transmission. Hence, the prevention of mother-to-child transmission of HBV plays a key role in the reduction of the disease prevalence. In many African countries, there is no active program to prevent mother-to-child transmission (PMTCT) of HBV. For a number of years, to prevent hepatitis B virus infection in children, the Ministry of Health of Burkina Faso, through the Expanded Program of Immunization (EPI), advocated the vaccine against HBV for all children at eight weeks of birth. This is already a strong prevention program but it would be necessary to vaccinate children from birth to block vertical transmission of the virus. Efforts to reduce significantly not only HBV infection in the general population but also its vertical transmission remain one of the global priorities in the fight against this public health disease.

Vaccination efforts have already been made: Collenberg *et al.*<sup>11</sup> had observed AbHBs levels of 69.6% and 76.4% respectively in Nouna and Ouagadougou. Some studies have shown that immunoprophylaxis through hepatitis B vaccine with or without Hepatitis B Immunoglobulin G (HBIG) is effective in preventing the transmission of HBV infection to infants: Milne *et al.*<sup>12</sup> (seroprotection rates = 98.0-98.6%); Zhu *et al.*<sup>13</sup> (seroprotection rates = 93.61%); van den Ende *et al.*<sup>14</sup> (seroprotection rates  $\geq 96.0\%$ ). A Brazilian study has also shown that administration of the hepatitis B vaccine at birth is immunogenic with a good safety profile in newborns.<sup>15</sup> As in this Brazilian study, and according to the transmission modes of HBV, it would be desirable to vaccinate newborns at birth to obtain data on the immunoprophylaxis of hepatitis B vaccine in Burkina Faso.

The main objective of this research, which was to prevent mother-to-child transmission of hepatitis B virus in Burkina Faso, has three specific objectives: i) to screen pregnant women for HBV infection; ii) vaccinate all children at birth against HBV regardless their mother HBV status; and iii) evaluate after 7 months of birth the level of their AbHBs among babies who received HBV vaccine at birth.

## Materials and Methods

### Type and period of study

This is a descriptive cross-sectional study, which took place from September 2013 to June 2014 at Saint Camille Hospital of Ouagadougou (HOSCO).

### Sampling

The study involved 237 term pregnant

women and their newborns. All study participants gave their free written and informed consent according to the Helsinki Declarations. Four milliliters of venous blood were collected from pregnant women for HBV screening. Seven months after delivery, venous blood samples were collected from 102 children previously vaccinated against HBV. These samples were used to evaluate AbHBs titer serological tests and confirmation using real time PCR in children born to HBV positive mothers.

### HBV screening

The HBV Combo Rapid Test Cassette Kit (Ref. No: IHBSG-401 Lot No.: HBSG14120001) was used for the rapid diagnosis of HBsAg and other markers of hepatitis B (HBeAg; AbHBe, AbHBs and AbHBc) were then determined in HBsAg-positive women.

### Newborns vaccination

One hundred and two (102) newborns received the first dose of the HBV vaccine (EUVAX-B® Pediatric) at birth, followed by the second and third doses at two and four months respectively. All vaccinated children were regularly followed up throughout the study period to evaluate side effects and other adverse reactions.

### Assay for antibody titer

The AbHBs titer was determined in children using the ELISA "AbHBs Quantitative EIA" Kit. It was obtained by averaging the concentration of three assays for each sample.

### Molecular diagnosis of HBV

Extraction of HBV DNA was performed using the "Genomic DNA Express" kit (Sacace Biotechnologies, Como, Italy). The HBV DNA was amplified by using the HBV Real-TM Qual Kit on the SaCycler-96

Real-Time PCR v.7.3 (Sacace Biotechnology, Como, Italy).

### Statistical analysis

The data were analyzed using SPSS software version 17.0 (SPSS, Chicago, IL, USA) and Epi info version 7.0. The chi-square test was used for comparisons and the difference was considered statistically significant for  $P < 0.05$ .

### Ethical consideration

Approval for this study was obtained from the Ethics Committee on Health Research of Burkina Faso (Deliberation No. 2013-7-065 on July 11, 2013). Written informed consent was obtained from all participants.

## Results

### HBV status according to sociodemographic characteristics of pregnant women

According to the data in Table 1, the women in this study were mostly housewives (50.2%) with an average age of  $27.0 \pm 5.3$  years. Among the 237 pregnant women enrolled, 22 were positive for HBsAg or a prevalence of 9.3%. There was no statistically significant difference between the mean age of HBV seropositive ( $28.1 \pm 6.7$  years) and seronegative ( $26.9 \pm 5.1$  years) women ( $P = 0.309$ , Table 1).

### Molecular diagnosis of HBsAg+ mothers and their children, and vertical transmission of HBV

Among the 22 pregnant women positive for HBsAg, 21 were confirmed positive for viral hepatitis B by PCR (HBV DNA+) with a concordance rate of 95.4% (21/22).

**Table 1. HBV status according to socio-demographic characteristics of pregnant women.**

Pregnant women	N.	HBV-	HBV+	P-value
Housewives	119 50.2%	110 46.4%	9 3.8%	0.001
Private sector	42 17.7%	33 13.9%	9 3.8%	-
Schoolchildren/Students	41 17.3%	39 16.5%	2 0.8%	-
Salaried employee	35 14.8%	33 13.9%	2 0.8%	-
Total	237	215 90.7%	22 9.3%	0.001
Mean age (years)	237 $27.0 \pm 5.3$	215 $26.9 \pm 5.1$	22 $28.1 \pm 6.7$	0.390

**Table 2. HBV PCR results in HBsAg+ mothers and their children (Seven months after delivery) and Vertical Transmission of HBV.**

HBV-positive mothers	Children	HBsAg	HBeAg	AbHBs	AbHBe	AbHBc	HBV DNA	HBV vertical transmission
Number of mothers	HBV DNA							
1	+	+	+	-	-	+	+	1
4	+	+	-	-	+	+	+	4
11	+	+	-	-	+	+	-	0
4	+	+	-	-	-	+	-	0
1	-	+	-	-	-	-	-	0
22								5

**Table 3. The rate of AbHBs in vaccinated children seven months after delivery.**

AbHBs concentration (IU/L)	N	AbHBs titer in children (IU/L)	Immune status
X <sup>&lt;11</sup> *	4/102	5.4 ± 4.1	Non-immune, 3.9%
X <sup>&gt;11</sup>	98/102	227.1 ± 61.5	Immune, 96.1%
Total	102	218.1 ± 74.7	

X: concentration; \* t-TEST: P<0.001

Among these 21 HBV DNA+ women, five (5) transmitted the infection to their children with a vertical transmission rate of 23.81% (5/21) (Table 2). These HBV-positive women have been referred to the specialist for medical care.

### Determination of anti-HBs antibody level in vaccinated children

The average concentration of HBsAg from the 102 children was 218.1±74.7 IU/L. A total of 98/102 or 96.1% of children were immunized with an average AbHBs concentration above 11 IU/L (227.1±61.5 IU/L) compared to 4/102 or 3.9% of non-immunized with an average concentration of AbHBs less than 11 IU/L (5.4±4.1 IU/L) (Table 3).

## Discussion

In this study, 9.3% (22/237) of pregnant women were HBsAg positive. This prevalence is similar to the 9.3% and 9.8% reported by Simpoire *et al.*,<sup>3,4</sup> respectively in 2004 and 2006 among pregnant women in Burkina Faso. However, it is lower than 12.04% and 11.4% respectively found by Ilboudo *et al.*,<sup>8</sup> and Sangare *et al.*,<sup>7</sup> in Ouagadougou and higher than those reported by Rouet *et al.*<sup>16</sup> in Ivory Coast (9.0%) and Maclean *et al.*,<sup>17</sup> in Northern Mali (8%) among pregnant women. These results confirm the high endemicity of hepatitis B in the West African sub-region. This difference in the prevalence could probably be due to the samples size and especially the various distribution of HBV by countries and geographical areas. For example, the low rate of screening for HBV in pregnant

women is a major risk factor for vertical transmission in sub-Saharan Africa countries.

Real-time PCR was performed to investigate the presence of viral DNA in the 22 HBsAg-positive mothers and their children. Twenty-one (21) mothers and five (05) children had HBV DNA corresponding to a vertical transmission rate of 23.8% (5/21).

The rate of HBeAg was 4.5% among mothers who transmitted the virus to their children. This rate is lower than 18.2%, 11.1% and 31.4% respectively found by Nacro *et al.*<sup>18</sup> in Bobo Dioulasso, Ilboudo *et al.*<sup>8</sup> and Sangare *et al.*,<sup>7</sup> in Ouagadougou, Burkina Faso. Indeed, previous studies reported that the presence of HBeAg in a pregnant woman is a significant risk factor for vertical transmission of HBV with a probability of 90% that the child become a chronic carrier.<sup>9,19</sup> Mother-to-child transmission routes of HBV vary by geographic area.<sup>20</sup> In this study, mothers who transmitted HBV to their children were positive for HBsAg, AbHBc, viral DNA, or HBeAg. Indeed, studies conducted by Walz *et al.*,<sup>21</sup> and Ilboudo *et al.*,<sup>22</sup> showed that vertical transmission was possible for a mother positive for AbHBc, HBeAg and viral DNA, respectively. These factors could explain the vertical transmission of HBV in the present study. Our results suggest a high risk of vertical transmission when pregnant woman is positive for HBsAg, AbHBc, HBeAg, and viral DNA in sub-Saharan Africa, mainly in Burkina Faso.

Combined intramuscular hepatitis B vaccines have been studied in different countries.<sup>23,24</sup> The immunogenicity of the three doses of EUVAX-B® intramuscular vaccine has already been confirmed in a

previous study with protection rate of 89.5%.<sup>25</sup> However, the present study is one of the first to demonstrate the immunogenicity and safety of intramuscular vaccine against hepatitis B at birth in Burkina Faso. These results from the three-dose series of primary hepatitis B vaccination from birth showed an immunization rate of 98.3% (AbHBs>11 IU/mL) in children seven months after vaccination. No adverse effects were reported in children throughout the study period. These results are consistent with those reported by Lee *et al.*<sup>26</sup> and especially Carniel *et al.*,<sup>15</sup> in Brazil who have shown the safety and immunogenicity of this kind of vaccination in newborns.

These results could contribute to the development of a new kind of neonatal vaccine co-formulation algorithm to prevent significantly HBV, BCG, and Poliomyelitis virus infections in neonates. However, the lack of routine screening of pregnant women, incomplete vaccination coverage or delayed vaccine delivery are among other major risks to consider in reducing vertical transmission of HBV.

## Conclusions

Vaccination remains a major tool for preventing and controlling the transmission of the hepatitis B virus. This study confirms that vertical transmission of HBV is health issues in our country and that vaccination from birth would significantly reduce this transmission. These results could contribute to the development of a new strategy in the Expanded Program on Immunization (EPI) to prevent significantly the vertical transmission of HBV infection in Burkina Faso.

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