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*Original Article*

# Hepatitis B vaccination in Burkina Faso: prevalence of HBsAg carriage and immune response in children in the western region

Armel Moumouni Sanou[1], Abdoul Kader Ilboudo[1], Ziemlé Clément Meda[2], Augustin Togozia[1], Abou Coulibaly[3], Assana Cissé[1], Tani Sagna[1], Kania Dramane[4], Zékiba Tarnagda[1]

*[1] Unité des maladies à potentiel épidémique, maladies émergentes et zoonoses, Institut de Recherche en Sciences de la Santé (IRSS), Bobo-Dioulasso, Burkina Faso*
*[2] Institut Supérieur des Sciences de la Santé (INSSA), Université NAZI BONI, Burkina Faso*
*[3] Département des sciences biomédicales et santé publique, Institut de Recherche en Sciences de la Santé (IRSS), Ouagadougou, Burkina Faso*
*[4] Département des Sciences Biomédicales, Centre Muraz, Bobo-Dioulasso, Burkina Faso*

## Abstract

Introduction: Hepatitis B virus (HBV) infection remains a major health problem in Burkina Faso. To control and prevent HBV infection, Hepatitis B vaccine was introduced in the national expanded program in 2006. In this study, we evaluated the prevalence of HBsAg in children aged under 10 years after one decade of universal hepatitis B vaccination, and the immune response among these children.

Methodology: Between May and October 2015, a cross-sectional study was conducted among children in two primary healthcare centers in the western region of Burkina Faso. Participants were enrolled in Accart-Ville Healthcare Center in Bobo-Dioulasso (urban area) and the Healthcare Center of the village of Djigouera (rural area). Blood samples were collected from all children and analysed for the presence of HBsAg and anti-HBs antibodies (Abs). For HBsAg positive children, blood samples were also taken among their mothers for screening for HBsAg.

Results: A total of 265 children were included in this study. The mean age was 4.4 years. HBsAg was found in 3.4% (9/265) of children. Of the 9 HBsAg positive children, 5 had HBsAg positive mothers. From the 265 children tested for quantification of anti-HBs Ab titer, 219 (82.6%) were fully vaccinated and 135 (61.6%) of them had an anti-HBs ≥ 10 mIU/mL.

Conclusion: Despite a good vaccination coverage (82.6%), a considerable proportion of vaccinated children remains unprotected from HBV infection. That emphasizes the need for further strengthening of the vaccination program through implementing the birth dose of HBV vaccine as recommended by WHO.

**Key words:** Hepatitis B vaccine; children; HBsAg; anti-HBs Abs; Burkina Faso.

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## Introduction

Hepatitis B virus (HBV) infection remains a major global health problem with 240 million chronic carriers and 780 000 deaths each year [1]. The burden of the disease is highest in sub-Saharan Africa and East Asia, where more than 8% of the adult population are chronically infected [1,2]. In these areas, the most common routes of HBV infection are perinatal transmission from mother to child at birth and horizontal transmission from close contacts during early childhood [3,4]. To control and prevent HBV infection, active immunization with hepatitis B vaccine remains the most effective measure. Indeed, a safe and effective vaccine against hepatitis B, available since 1982, prevents HBV infection when it is given before or shortly after exposure. Regardless of country-specific prevalence, the World Health Organization (WHO) has recommended at least three doses of hepatitis B vaccine for all infants, including a first dose within 24 hours of birth [5]. Burkina Faso is an endemic area for HBV infection with an at least 10% prevalence of Hepatitis B surface antigen (HBsAg) carriers [6-8]. Hepatitis B vaccine was introduced in the national expanded program on childhood immunization in 2006. The HBV vaccine is used as a combined vaccine (pentavalent vaccine: DTC-HepB-Hib1) administrated at months 2, 3 and 4 respectively. However, factors such as genetic make-up, immunosuppression, vaccine storage conditions, obesity, diabetes, and gender have been implicated to affect the immune response to hepatitis B vaccination [9,10]. To our knowledge, only one study assessed the effectiveness of Hepatitis B

vaccine in children in Burkina Faso [11]. This study has focused on children aged from 6 – 18 months living in the city of Ouagadougou, an urban setting located in the central part of the country. No data were available in the western part of Burkina Faso and still little in a rural area. To address this gap, we evaluated (i) the prevalence of HBsAg in children aged under 10 years after one decade of universal hepatitis B vaccination in western region of Burkina Faso, and (ii) the immune response among these children.

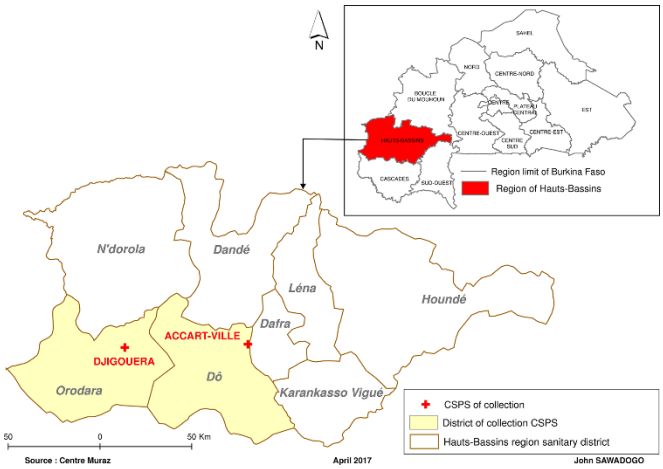
## Methodology

*Study design*

From May to October 2015, we conducted a cross-sectional study in two primary healthcare centers in the western region of Burkina Faso (Figure 1). Participants were children < 10 years old, attending care or vaccination, with an immunization or health card, and from which we obtained a written informed consent of the parents or legal guardian after careful explanation. Therefore, children who received vaccine outside Burkina Faso were not included in the study. A total of 215 children were to be enrolled. This estimation was calculated basing on a proportion of 85% of children with an anti-HBs level ≥10 mIU / mL [5] and using Schwartz's formula. Children were randomly selected to participate based on the immunization or health card. The procedure was followed until the requested sample size was reached. Participants were recruited in the Accart-ville Healthcare Center in Bobo-Dioulasso city (urban area) and the Healthcare Center of Djigouera (rural area).

For the ethical considerations, the study was approved by the Regional Health Authority, which is responsible for the implementation of health policies and ensuring patient safety in the study area. Written informed consent was obtained from the mother or legal guardian of all children prior to enrolment. HBsAg positive children were referred to a hepatitis specialist for a better clinical management with the support from a hepatitis fighting organization located in Bobo-Dioulasso.

**Figure 1.** Map of the study area.




*Data collection*

Demographic characteristics (e.g. age, gender, place of birth, level of education, etc.) and hepatitis B vaccination history were collected using a structured questionnaire.

*Specimen collection and laboratory testing*

From each child ≥ 2 months old, blood samples were taken under aseptic conditions in red vacutainer tubes and the sera obtained after centrifugation was stored at – 80°C until laboratory analysis. From participants under 2 months old, whole blood was collected onto Whatman filter paper (Whatman no. 903; formely SS903, Schleicher & Schull, Kenne, NH, USA) since venepuncture is arduous in this age group. At least 3 spots (50 µL each) of each filter paper card were eluted in 600 µL of phosphate-buffered saline (PBS) at 4°C overnight. All specimens for all the children were tested for the presence of HBsAg using a point of care testing (First Response HBsAg Card Test, Premier Medical Corporation, Kachigam, India) and for antibodies against Hepatitis B surface antigen (anti-HBs Abs) by Enzyme Linked Immunosorbent Assay (ELISA) (Bio-Rad, Marnes-La-Coquette, France). Children with anti-HBs Ab titer ≥ 2 mIU/mL were considered as seroconverted and those with anti-HBs Ab titer ≥ 10 mUI/mL as protected against HBV infection. For HBsAg positive children, blood samples were also taken from their mothers for HBsAg screening. All HBsAg positive samples were confirmed by Architect Ci 4100 analyser (Abbott, Palm City, USA). All tests were performed and interpreted in accordance with the manufacturers' instructions. Analyses were carried out in the virology laboratory of "Institut de Recherche en Sciences de la Santé" (IRSS) in Bobo-Dioulasso, Burkina Faso.

*Data management and analysis*

We used Chi-square and Fisher exact tests to compare HBsAg presence or absence according to children characteristics when appropriate. HBsAg prevalence rates were expressed in percentages with 95% confidence intervals (95% CI). Odd ratios and their 95% confidence intervals were also calculated to determine association between anti-HBs Ab

concentration and demographic characteristics of subjects. Data were entered and checked for accuracy using Epidata software version 3.1. All data analyses were performed using STATA version 14.0 (StataCorp, College Station, Texas, USA). For all analyses, we considered a p-value ≤ 0.05 as statistically significant.

## Results

*General characteristics of the study population*

Overall, 265 children aged < 10 years (mean age: 4.4 years; range: 1 month – 9 years) were tested for markers of HBV infection. Of these, 167 (63.0%) were from an urban area (Bobo-Dioulasso), 143 (53.9%) were male with a gender ratio of 1.2, 220 (83.0%) were aged 1 – 9 years and 233 (87.9%) received at least one dose of HBV vaccine. The proportion of those who were born in a healthcare facility was 87.5% (232/265). Sociodemographic characteristics of the study population are summarized in Table 1.

*Prevalence of HBs Antigen*

Characteristics of HBsAg carriers are described in Table 2. The overall prevalence of HBsAg in this study population was 3.4% (9/265; CI 95%: 1.7% - 6.4%). Among participants from the rural area, the HBsAg prevalence was significantly higher (7.1%; CI 95%: 3.4% - 14.3%) than among urban participants (1.2%; CI 95%: 0.3% - 4.7%), (p = 0.010). When stratified by age, the prevalence was lower in children aged under 5 years (0.7%; CI 95%: 0.3% - 14.5%) but increased in children aged from 5 – 9 years (6.8%; CI 95%: 3.4% - 13.1%), (p = 0.018). For other characteristics such as birth place, mother's education level and vaccination, no statistically significant differences were found (Table 2). Of the 9 children who were tested HBsAg positive, 5 had mothers who were positive for HBsAg. All of them were from Djigouera.

*Anti-HBs antibody titer*

Of the 265 children tested for anti-HBs Abs, 219 (82.6%) were fully vaccinated and 135 (61.6%) of them had an anti-HBs ≥ 10 mIU/mL (Table 3). Of the 246 children who have reached the age to receive all vaccine doses, 30 (12.2%) were missing some and 22 (8.9%) received no dose.

Statistical analysis showed a positive association between antibody concentration and residence in an urban area (OR = 1.9; IC 95%: 1.1 – 3.2; p = 0.011) (Table 3). Furthermore, children aged 1 - 5 years are more protected than other age groups (OR = 2.2; CI 95%: 1.1 – 4.6; p = 0.0065).

**Table 1.** Sociodemographic characteristics of the study population.

| Characteristics | Healthcare Center of Accart-Ville (Urban area) n = 167 (%) | Healthcare Center of Djigouera (Rural area) n = 98 (%) | Total N = 265 (%) |
|---|---|---|---|
| **Gender** | | | |
| Male | 84 (50.3) | 59 (60.2) | 143 (53.9) |
| Female | 83 (49.7) | 39 (39.8) | 122 (46.0) |
| **Age groups (year)** | | | |
| < 1 | 38 (22.7) | 7 (7.1) | 45 (17.0) |
| 1 – 5 | 85 (50.9) | 18 (18.4) | 103 (38.9) |
| > 5 | 44 (26.3) | 73 (74.5) | 117 (44.1) |
| **Birth place** | | | |
| Health center | 163 (97.6) | 69 (70.4) | 232 (87.5) |
| Home | 4 (2.4) | 29 (29.6) | 33 (12.4) |
| **Mother's education level** | | | |
| Unschooled | 74 (44.3) | 98 (100.0) | 172 (64.9) |
| Elementary school | 34 (20.4) | 0 (0.0) | 34 (12.8) |
| Secondary school | 51 (30.5) | 0 (0.0) | 51 (19.2) |
| University | 8 (4.8) | 0 (0.0) | 8 (3.0) |
| **Vaccination status** | | | |
| Vaccinated | 158 (94.6) | 75 (76.5) | 233 (87.9) |
| Unvaccinated | 9 (5.4) | 23 (23.5) | 32 (12.1) |
| **Dose received** | | | |
| Zero dose | 9 (5.4) | 23 (23.5) | 32 (12.1) |
| One dose | 5 (3.0) | 2 (2.0) | 7 (2.6) |
| Two doses | 5 (3.0) | 1 (1.0) | 6 (2.3) |
| Three doses | 148 (88.6) | 72 (73.5) | 220 (83.0) |

**Table 2.** Characteristics of HBsAg carriers.

| Characteristics | HBsAg (-) N = 259 (%) | HBsAg (+) N = 9 (%) | *p* value |
|---|---|---|---|
| **Gender** | | | |
| Male | 138 (96.5) | 5 (3.5) | 0.922 |
| Female | 118 (96.7) | 4 (3.3) | |
| **Residence** | | | |
| Bobo-Dioulasso (urban area) | 165 (98.8) | 2 (1.2) | 0.010 |
| Djigouera (rural area) | 91 (92.9) | 7 (7.1) | |
| **Age groups (year)** | | | |
| < 1 | 44 (97.8) | 1 (2.2) | |
| 1 – 5 | 103 (100.0) | 0 (0.0) | 0.018 |
| > 5 | 109 (93.1) | 8 (6.8) | |
| **Birth place** | | | |
| Health centre | 225 (97.0) | 7 (3.0) | 0.366 |
| Home | 31 (93.9) | 2 (6.1) | |
| **Education level of mother** | | | |
| Unschooled | 163 (94.8) | 9 (5.2) | |
| Elementary school | 34 (100.0) | 0 (0.0) | 0.169 |
| Secondary school | 51 (100.0) | 0 (0.0) | |
| University | 8 (100.0) | 0 (0.0) | |
| **Vaccination status** | | | |
| Vaccinated | 226 (97.0) | 7 (3.0) | 0.342 |
| Unvaccinated | 30 (93.8) | 2 (6.2) | |

**Table 3.** Seroconversion and seroprotection status (anti-HBs Abs).

| Characteristics | Anti-HBs Abs N = 265 | | OR (IC 95%) | *p* value |
|---|---|---|---|---|
| | < 10 IU/mL n = 106 (%) | > 10 IU/mL n = 159 (%) | | |
| **Gender** | | | | |
| Male | 59 (41.3) | 84 (58.7) | 1 | - |
| Female | 47 (38.5) | 75 (61.5) | 1.1 [0.7 – 1.8] | 0.651 |
| **Residence** | | | | |
| Bobo-Dioulasso (urban area) | 57 (34.1) | 110 (65.9) | 1.9 [1.2 – 3.2] | 0.011 |
| Djigouera (rural area) | 49 (50.0) | 49 (50.0) | 1 | - |
| **Age groups (year)** | | | | |
| < 1 | 21 (46.7) | 24 (53.3) | 1 | - |
| 1 – 5 | 29 (28.2) | 74 (71.8) | 2.2 [1.1 – 4.6] | 0.030 |
| > 5 | 56 (47.9) | 51 (52.1) | 0.9 [0.6 – 2.0] | 0.891 |
| **Dose received** | | | | |
| Zero | 14 (43.7) | 18 (56.2) | 1 | |
| One dose | 6 (85.7) | 1 (14.3) | 0.1 [0.0 – 1.2] | 0.072 |
| Two doses | 2 (33.3) | 4 (66.7) | 1.5 [0.2 – 9.7] | 0.637 |
| Three doses | 84 (38.2) | 135 (61.6) | 1.2 [0.6 – 2.7] | 0.547 |

No significant association was found between immune status and variables such as gender, vaccination, number of doses received ($p > 0.05$) (Table 3). Eighteen children who received no HBV vaccine had an anti-HBs titer greater than 10 mUI/mL.

## Discussion

This study investigated for the first time the impact of universal Hepatitis B vaccination in the western region of Burkina Faso since its implementation in 2006. For this, the seroprevalence of HBsAg and the seroprotection status were evaluated in children born after HBV vaccine introduction. The overall HBsAg positivity was high (3.4%) in our study even though it was lower than reported in previous studies conducted in Burkina Faso that showed a prevalence rate of 4.1% in new borns in 2005 and 12.2% in children in 2001 [12,13] respectively. It was low in younger children ≤ 5 years (0.7%). However, it increased with age to reach a prevalence of 6.5% in children ≥ 5 years. The high prevalence among children aged 5 to 9 years in our study population supports the role of the mother-to-child transmission and early high horizontal transmission. Indeed, five mothers of children tested positive for HBsAg were also positive with a risk of transmission during the perinatal period, depending on the level of maternal HBV viral load. In addition, 7 of the infected children were fully immunized, suggesting that they were already infected when receiving doses of HBV vaccine. These results once more highlight the evidence of mother-to-child transmission of HBV in sub-Saharan Africa and the urgent need for the birth-dose of HBV vaccine.

Analysis of the sociodemographic data reveal that the residence in a rural area is a significant risk factor for acquiring HBV infection. Participants from a rural area are six fold more exposed than urban participants (OR = 6.3; CI 95%: 1.3 – 31.2; p = 0.023). This situation could be due to the low health facilities attendance (delivery, vaccination, etc.) related to a lower socioeconomic status in a rural area. This finding is in line with previous studies in Sub-Saharan Africa and elsewhere which reported higher prevalence of HBV infection in rural settings compared to semi-urban or urban settings [14-18].

A low proportion (61.6%) of fully vaccinated children had an anti-HBs titer ≥ 10 mUI/ml indicating their immunity against HBV infection. Furthermore, in children with an anti-HBs titer < 10 mUI/ml, 68 (81%) of them were aged over 18 months of age suggesting a rapid wane of the anti-HBs titer after vaccination. Our results are consistent with most studies carried out in Sub-Saharan Africa settings [19-21]. In contrast, Ouedraogo et *al*. found a seroprotection of 90% in children in Ouagadougou [11] which is close to the high vaccine-induced HBV-protection rate (90–95%) observed among children living in western countries [22]. The difference between our results and those from Ouedraogo et *al*. could be due to the characteristics of the study population including especially the age of subjects since anti-HBs titer decline over time [23]. Indeed, Ouédraogo et *al*. have worked in a population from an urban setting (Ouagadougou) with an age group of 6 – 18 months. Statistical analysis in our study revealed that anti-HBs level ≥ 10 mUI/ml was positively associated to age between 1 – 5 years (OR: 2.2; CI 95%: 1.1 – 4.6; p = 0.03) and residence in an urban area (OR: 1.9; CI 95%: 1.2 – 3.2; p = 0.011).

The low level of immunity in fully vaccinated children or non-responder children may be explained by several other reasons. First, the vaccine quality may be compromised by inappropriate storage conditions. In Burkina Faso where refrigerators for the storage of vaccines run with electricity, the cold chain is disrupted by frequent power outages. In addition, in rural settings where the cold chain operates using butane gas, problems of supply are common. Second, the nutritional status of children could have an impact on antibody response to vaccination [24]. Malnutrition is a public health problem in Burkina Faso and it is the second most common cause of morbidity in children under five years with 30.2% of undernourished children [25]. Third, with about 13 000 children [26] living with HIV in Burkina Faso, this could be another factor explaining the low immunity of HBV vaccine in fully vaccinated children.

Eighteen unvaccinated children (56.2%) have produced a protective anti-HBs Ab level, suggesting acquisition of natural immunity. Unfortunately, as a limitation of our study, it was not possible to test for antibodies against hepatitis B core antigen (anti-HBc) which is a marker of past infection.

Other main limitations in our study are related to the low sample size that did not allow us to extrapolate results to the general population of children aged 0 to 10 years. Finally, data on nutritional status were not available making the interpretation of the results difficult, especially in non-responders to the vaccine.

## Conclusion

This study showed a good vaccination coverage (82.6% of children were fully vaccinated) but a prevalence of HBsAg (3.40%) in children was observed. In addition, a considerable proportion

(38.2%) of fully vaccinated children remains unprotected to HBV infection 10 years after including HBV vaccine in the expanded program of immunization of Burkina Faso. That emphasizes the need of further strengthening the vaccination program in Burkina Faso through implementing the first dose of HBV vaccine within 24 hours of birth as recommended by WHO.

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## Author's contributions

AMS, ZCM and ZT designed the study and drafted the protocol. AMS, AKI and AT collected the samples and compiled the epidemiological data. The laboratory analysis and interpretation of the results were performed by AMS, AT, AC3 and TS. AMS and AC1 analyzed the data. DK contributed for reagents. AMS, AKI and DK wrote the paper. All authors read and approved the final manuscript for publication.

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## Corresponding author

Armel Moumouni Sanou
Institut de Recherche en Sciences de la Santé (IRSS), 399 Avenue de la liberté, BP 545, Bobo-Dioulasso, Burkina Faso
Tel : (00226) 71 13 19 12
Fax: (00226) 20 98 18 80,
E-mail: armelbf@gmail.com

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