



## ARTICLE

# Protection level of anti-hepatitis B vaccine and immunoglobulin in a pediatric Cameroonian population

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## Abstract

**Background:** Despite nearly twenty years of an effective vaccine, hepatitis B remains one of the most common viral infections worldwide. Mother-to-child transmission is a major route in children.

**Objective:** to evaluate HBV-infected mothers' children's vaccination response.

**Methods:** The Centre Hospitalier Dominicain St-Martin de Porres, Yaounde prenatal care (ANC) database included HBsAg-positive consenting women and their children. Social demographics were acquired using a tested questionnaire. The 5 hepatitis B indicators were evaluated and anti-HBsAg antibodies quantified by indirect ELISA. Microsoft Excel and Epi-info was used to examine data.

**Results.** Out of 5,996 women registered, 143 were HBsAg positive (2.38% prevalence) and none were HBeAg positive. Of 143 HBsAg-positive women, 50 participated in the study. Out of 50 positive moms, 78 children were included, with a mean age  $\pm$  SD of  $2.33 \pm 2.86$  years. None of the children were infected with HBV, but all were exposed. 64 (82.05%) received anti-HBs immunoglobulin (HBIG) and a vaccine at birth, while 14 (17.95%) received only the vaccine. 72 (92.31%) children received all three vaccination doses. The vaccine responders were 62.82% (above 10 IU/mL), while 37.18% of children were non-responders, a greater risk group if not supplemented.

**Conclusion:** This study found 92.31% child anti-HBV immunization coverage. WHO recommends 95% protection, but 62.82% is below that. The causes of this poor protection should be examined.

**Keywords:** Cameroon, pediatric patients, anti-hepatitis B, vaccines.

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## 1 | INTRODUCTION

**H**epatitis B is a liver disease caused by the HBV virus, capable of establishing acute infections, acute liver failure as well as chronic infections in humans, which can be potentially lethal. In 2015, WHO estimated that 257 million people were living with chronic hepatitis B (defined as positive hepatitis B surface antigen). That same year hepatitis B caused an estimated 887,000 deaths, mainly from cirrhosis or hepatocellular carcinoma. It is a highly endemic condition in sub-Saharan Africa and approximately 63 million new HBV cases have been estimated to occur between 2015 and 2030 [1].

The World Health Organization proposes to achieve the goal of eliminating the public health threat of viral hepatitis by 2030, and the key to achieving this ambitious goal is to effectively block hepatitis B through standardized and refined management of pregnant women and their newborns [2]. Vaccination of women of childbearing age is one of the major pillars of prevention of vertical transmission of HBV. Maternal-fetal transmission of HBV represents an essential link in the maintenance of the infection, especially in highly endemic countries. In the absence of any preventive interventions, the risk of transmission from mother to child ranges from 70% to 90% for mothers with high HBV viral load (or are HBeAg positive) and from 10% to 40% for those that are HBeAg negative.

High concentrations of HBV DNA (viral load) are associated with an elevated risk of transmission, even among infants who receive the hepatitis B vaccine. Thus these pregnant women should be administered antiviral prophylaxis to prevent mother-to-child transmission [3]. When the mother tests positive for HBs antigen (Ag), the newborn must receive an intramuscular injection of anti-HBs immunoglobulin (HBIG) at two different sites of injection, then the first dose of vaccine at birth. Vaccination is then continued according to a 3-injection schedule (4 in the case of premature infants, see Table 1). The final dose of the vaccine series should not be given before 24 weeks (164 days) of age. For preterm infants weighing less than 2 kg, the initial dose of vaccine (birth dose) should not be included in the vaccine

series because of the potentially reduced immunogenicity of the HBV vaccine in these infants; three additional doses of vaccine (for a total of four doses) should be given starting when the infant reaches 1 month of age [4]. However, the effectiveness of HBV transmission prevention must be assessed by the determination of the proportion of infected (HBsAg positive) children; while the measurements of anti-HBs antibodies 1–2 months after completing the hepatitis B vaccine series allows to determine the rate of infants who responded adequately to the vaccine and have a protective level of antibodies (>10 IU/ml). Hence the justification of this cross-sectional study, aiming to evaluate the effectiveness of the protective vaccine in children born to HBV positive mothers in Cameroon.

## 2 | METHODS

### Study design and settings

In the database of the ante-natal consultation service (ANC) of the Dominican Hospital Center Saint Martin de Porres (CHDSMP) in Yaounde, mothers who gave birth between January 1 and November 30, 2020 were recorded and their HBV status noted. Those HBsAg positive were contacted to participate, and received information on the objectives of the study. The CHDSMP was selected because of its high rate of frequentation by pregnant women, where, hepatitis B screening is routinely performed in parturients.

### Study population

The study population consisted of mothers who were HBsAg positive during ANC and their child(ren). The sampling technique was the non-probability convenience technique. In addition, only those who agreed to participate in our study were included.

**Supplementary information** The online version of this article (<https://doi.org/xx.xxx/xxx.xx>) contains supplementary material, which is available to authorized users.

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### Ethical considerations

Administrative authorization from CHDSMP and ethical approval from the Health Sciences School of Catholic University committee were obtained (CEIRSH n° 2020/02008/CEIRSH/ESS/MIM). Informed parental consent was obtained from all participants.

Sociodemographic parameters were collected from participants using a structured questionnaire.

### HBV testing

Plasma collected from children was used in a rapid test for the diagnosis of the 5 HBV markers (HBsAg, HBeAg, HBeAb, HBsAb and HBcAb) following manufacturer instructions (HIGHTOP Biotech Co., Ltd., Qingdao City, 266112, Shandong Province, China). A test cassette was labelled with the code assigned to each child. Three drops of plasma were deposited at the corresponding site on the cassette. After the development of the reaction, the results were read y 15 minutes after sample deposition. A positive result was defined by the presence of a revelation band on the control area as well as on the patient area.

### Quantification of anti-HBs antibodies in children

The quantification was done using the antigen sandwich ELISA method according to the manufacturer instructions (HBsAb quantitative ELISA kit cat MID 0010, Melsin Medical CO; Ltd-Changchun; China). The optical density was measured, and was proportional to the amount of antibody captured in the wells.

### Statistical analysis

Data were recorded in Microsoft Excel 2016 software. Numbers, frequencies, means, medians, odd ratios and p-values were calculated by Epi info V.7 software. All p values <0.05 were considered statistically significant. Graphical representations were performed by Microsoft Excel 2016 software.

## 3 | RESULTS

### HBV prevalence in mothers

From January 1<sup>st</sup> to November 30<sup>th</sup>, 2020, 5,996 pregnant women were registered in ANC record

book of the CHDSMP, among whom 143 were diagnosed positive for the HBsAg marker of HBV, giving a prevalence of 2.38%.

### HBV prevalence among their children

Of the 143 positive women contacted, 50 responded. Of these 50 mothers we were able to enrol 78 children (as some mothers brought back more than one child). These children were aged 2 months to 11 years. They were tested for the presence of HBsAg, and none of them was positive, making the prevalence of HBsAg in children of 0%, as presented in Table 2. All of them were positive to anti-HBe Ab (100%), while 76 (97.44%) were positive to anti-HBc Ab. The positivity to these antibodies testifies that these children have been in contact with the virus.

### Vaccine coverage and protection level

Using the qualitative test, 2 of the 78 children (2.56%) were negative for anti-HBs antibody and 76 of them (97.44%) were positive for anti-HBs Ab.

Of the 78 children tested, 64 (82.05%) received at birth both anti-HBs immunoglobulin (HBIG) and one dose of vaccine, while 14 (17.95%) received only one dose of vaccine at birth. 72 children out of 78 received all the three doses as recommended by the EPI (Table 3).

Of the 78 children, 62.82% had vaccine protection, as shown in Table 4.

The quantity of antibodies (from optical density) was analysed according to age. The Pearson's coefficient being negative, we observe the decrease of the quantity of anti-HBsAb according to the age of the children (see figure 1).

## 4 | DISCUSSION

### HBV prevalence in mothers

HBV infection in adults results in chronic hepatitis in less than 5% of cases, whereas in infants and young children, it causes the development of a chronic form of the disease in 95% of subjects [6]. Knowing the hepatitis B status of pregnant women is critical for effective disease management and prevention of mother-to-child transmission [7]. Prevalence rates of

hepatitis B among pregnant women varied considerably from one study site to another, ranging from 1.1 to 9.6% in Cameroon [8-10]. A review study reported in 2017 a prevalence of 9.8% among pregnant women [11]. In another study conducted in Tokombéré, 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 [12]; very high result compared to ours. This high rate could be due to a low knowledge of the disease and ethno socio-demographic characteristics in this region of Cameroon. In our study, a prevalence of 2.38 was found, lower than that of Fomulu in Yaounde. This could also show the trend in the reduction of HBV prevalence in the country between 2017 and 2020.

### Prevalence of HBV infection in children

No child born to HBV positive mothers in our study was HBsAg positive. Slightly different result than the one obtained in a study conducted in Thailand and lower than that reported in 2022 by Shimakawa et al [12] in Tokombéré, in the far North of Cameroon. They reported a residual risk of 5.6% among children born to HBV positive mother despite birth dose vaccine. Although the two studies were done in two different socio cultural settings, different study period, beside the birth dose vaccine, children of our study receive a dose of immunoglobulin. Could this immunotherapy justify the no infection or a bias due to small size population? Although HBsAg was negative, the existence of occult HBV infection needs further study. Three prospective studies were initiated in Thailand to investigate the immunogenicity, reactogenicity and efficacy of a recombinant hepatitis B vaccine in children born to mothers that had different seropositivity status of HBsAg and HBeAg. Only one subject out of the 76, who acquired HBV infection at birth, did not respond to primary vaccination and maintained a chronic condition throughout the long-term follow-up period. None of the other subjects reported clinical symptoms of HBV infection during the 20-year follow-up [5].

### Vaccine coverage and protection level

To prevent transmission of HBV from mother to child, the first dose of HBV vaccine should be given

as soon as possible after birth (preferably within 24 hours). The vaccine provides long-term protection against hepatitis B virus; however, protection is not immediate (it usually takes several weeks to develop antibodies to the virus). For this reason, this vaccine should be combined with hepatitis B immunoglobulin (HBIG). HBIG contains mainly immunoglobulin G (IgG) with a high affinity for the hepatitis B virus surface antigen (HBsAg) and has the same characteristics as the physiological anti-HBs antibodies. However, these passively administered immunoglobulins fade with a half-life of 22 days and are, if administered alone, provide only a temporary protection [13].

HBsAg-negative infants with anti-HBs levels of 10 mIU/mL or higher are protected and do not require additional medical management. HBsAg-negative infants with anti-HBs levels below 10 mIU/mL should be revaccinated with a second series of three doses and retested 1 to 2 months after the final vaccine dose. In the study carried out in Taiwan, of the 29 subjects included in the cohort of immunogenicity, three subjects had no detectable antibodies (<3.3 mIU/ml) before the administration of challenge dose. However, one month post-challenge dose, all subjects were found to be seropositive for anti-HBs antibodies. Among these subjects, an anamnestic response was observed in almost all subjects (n = 28/29; 96.6% (95% CI: 82.2–99.9) and 93.1% (95% CI: 77.2–99.2) had anti-HBs concentration  $\geq 100$  mIU/ml [5].

Following the above-mentioned recommendations, all children enrolled in this study received the vaccine against HBV. Most of them received hepatitis B immunoglobulin against this virus at birth. This may explain the zero rate of MTCT recorded. It also shows the cumulative effectiveness of these two methods of protection.

Over the past few decades, a series of long-term follow-up studies in vaccinated infants have established that universal immunization with hepatitis B vaccine from birth significantly reduced the subsequent development of chronic HBV infection in young adults following perinatal or early exposure [14]. These became apparent in terms of reduction not only in the incidence of acute clinical hepatitis B,

but also in HBsAg carrier status among successfully immunized populations.

In our study, the presence of anti-HBe and anti-HBc antibodies is evidence of exposure of these children to the hepatitis virus[15]. Thus, the viral particles would have been effectively neutralized by the action of HBIG (consisting mainly of anti-HBsAg antibodies), thus testifying the efficacy of the immunoserum. A study by Wang et al., 2016 [16] investigating the protective effect of improved vaccination practice against mother-to-child transmission of hepatitis B virus and risk factors associated with immunoprophylaxis failure was able to demonstrate that in a total of 863 mothers and their corresponding 871 infants, HBV transmission to infants born to HBeAg-negative mothers was successfully prevented. Based on the data in the literature, there are several cases of non-responders to the vaccine, consistent with the ELISA results of our study. Non-responders to the vaccine should be revaccinated.

#### **Proposal for non-responding subjects**

The results of our study showed a rate of 37.18% non-responders. This proportion of children are at risk of HBV infection.

A study in Italy support the current knowledge on the long-term persistence of anti-HBsAg antibody titers at seroprotective levels in subjects properly immunized against HBV according to the vaccination programs in Italy [17]. For those with an anti-HBs titer < 10 mIU/mL, a single dose of hepatitis B vaccine should be administered. In the same study, more than 95% of subjects “non-responder” to HBV vaccination, receiving an HBV vaccine booster dose demonstrated a seroprotective anti-HBs antibody titer after 2 weeks from vaccination.

Antibody responses to hepatitis B vaccine vary widely between individuals. 10-15% of adults fail to respond, or have a poor response. It is preferable to achieve anti-HBs levels above 100 mIU/mL. However, levels of 10 mIU/mL or more are generally accepted as enough to protect against infection.

**Limitation:** In addition to the small size population for this study, the kit used for antibody quantification may not have received WHO approval.

## **5 | CONCLUSION**

Hepatitis B, whose prevalence is quite high in Cameroon, represents a major public health problem. At the end of this study, whose main objective was to evaluate the prophylactic efficacy of vaccination, as well as the therapeutic efficacy of serovaccination received at birth by children of HBV positive mothers at the CHDSMP, it was found that the prevalence of HBsAg in mothers registered at the CHDSMP's ANC service is 2.3% and no cases of mother-to-child transmission were recorded. All children born to these HBV-positive mothers received the hepatitis vaccine and almost all received immunotherapy. This is to be encouraged and implemented in all other health facilities in Cameroon, the vaccination coverage in CHDSMP is particularly satisfactory, but the protection rate of 62.82% is suboptimal and required investigation.

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#### **Authors' contributions**

**MK** has collected the data, analyzed them, drafted the manuscript, participated in the revision, approved the submitted version, and agreed to be accountable. **RE** has analyzed the data, revised the manuscript, participated in the revision, approved the submitted version, and agreed to be accountable. **AN** has analyzed the data, drafted the manuscript, participated in the revision, approved the submitted version, and agreed to be accountable. **PFE** has collected the data, analyzed them, drafted the manuscript, participated in the revision, approved the submitted version, and agreed to be accountable. **CNN** has conceived and designed the study, analyzed the data, revised the manuscript, approved the submitted version, and agreed to be accountable.

**Ethics approval and consent to participate:** The study received the ethics approval of the All participants gave their consent and parental or assent when appropriate. All experimental protocols were approved by the “Comité d'éthique institutionnel de la recherche pour la santé humaine (CEIRSH) n°

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**Competing interests:** All authors declared no competing interest.

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**Table 1: Vaccination schedule for children aged 0 to 11 months in Cameroon.**

Contacts	Age	Vaccine	Route of administration	Preventable diseases
1st contact	At birth	BCG	Intradermal	Tuberculosis
		OPV 0	Oral	Poliomyelitis
2nd contact	6 weeks	DTP-HepB-Hib 1	Intramuscular	Diphtheria, Tetanus, Pertussis, Infection due to Haemophilus Influenzae type b, Hepatitis B
		OPV 1	Oral	Poliomyelitis
		Pneumo 13-1 (PCV)	Intramuscular	Pneumococcal infections
		ROTA 1	Oral	Rotavirus Diarrhoea
3rd contact	10 weeks	DTP-HepB-Hib 2	Intramuscular	Diphtheria, Tetanus, Pertussis, Infection due to Haemophilus Influenzae type b, Hepatitis B
		OPV 2	Oral	Poliomyelitis
		Pneumo 13-2	Intramuscular	Pneumococcal infections
		ROTA 2	Oral	Rotavirus Diarrhoea
4th contact	14 weeks	DTP-HepB-Hib 3	Intramuscular	Diphtheria, Tetanus, Pertussis, Infection due to Haemophilus Influenzae type b, Hepatitis B
		OPV 3	Oral	Poliomyelitis
		IPV	Intramuscular	Poliomyelitis
		Pneumo 13-3	Intramuscular	Pneumococcal infections
5th contact	6 to 11 months	Vit A	Oral	
	At 9 months	MR	Subcutaneous	Measles, Rubella
		YF	Subcutaneous	Yellow fever

Table 2: Distribution of the "Children" population according to the 05 HBV markers.

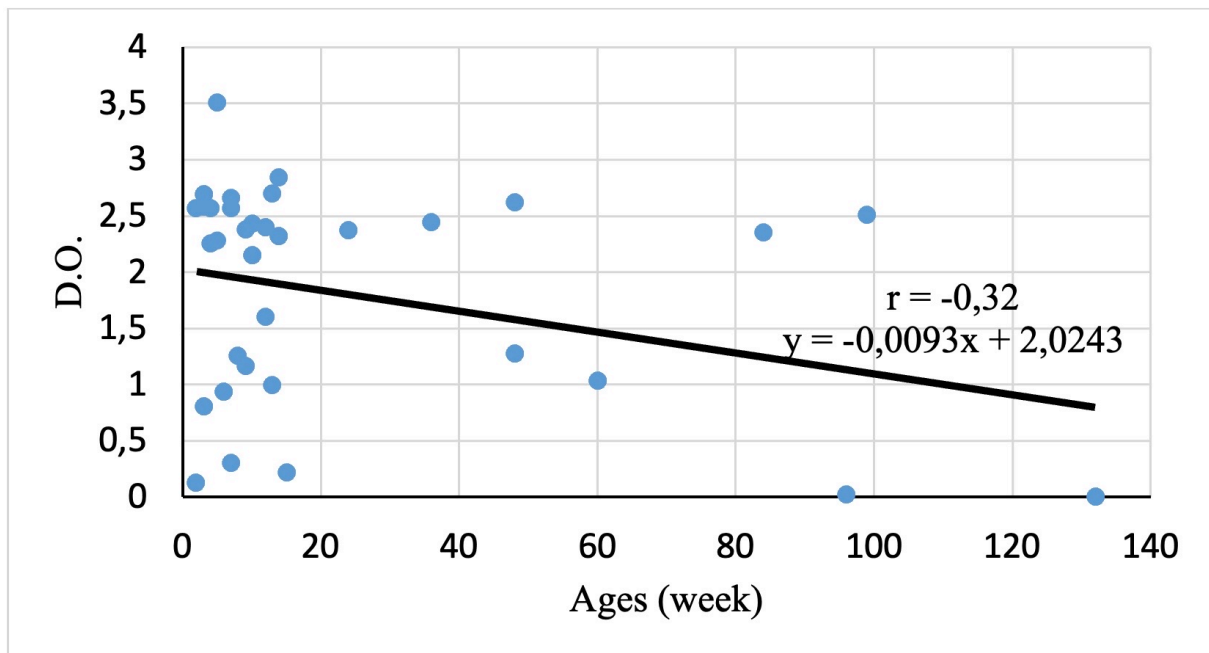
Age of new born	n (%)	HBsAg		Anti-HBsAb		HbeAg		Anti-HbeAb		Anti-HBcAb	
		N	P	N	P	N	P	N	P	N	P
0 to 2 months	5 (6.41%)	5 (100%)	0 (0%)	0 (0%)	5 (100%)	5 (100%)	0 (0%)	0 (0%)	5 (100%)	2 (40%)	3 (60.00%)
2 months to 1 yr	36 (46.15%)	36 (100%)	0 (0%)	0 (0%)	36 (100%)	36 (100%)	0 (0%)	0 (0%)	36 (100%)	0 (0%)	36 (100%)
1 year to 5 yr	26 (33.33%)	26 (100%)	0 (0%)	2 (7.69%)	24 (92.31%)	26 (100%)	0 (0%)	0 (0%)	26 (100%)	0 (0%)	26 (100%)
More than 5 yr	11 (14.10%)	11 (100%)	0 (0%)	0 (0%)	11 (100%)	11 (100%)	0 (0%)	0 (0%)	11 (100%)	0 (0%)	11 (100%)

N: Negative; P: Positive.

Table 3: Distribution of the children according to vaccine profile.

Administration		(n = 78)	Frequency
Received birth-dose of vaccine and HGIB	No	14	17.95%
	Yes	64	82.05%
Number of HBV vaccine doses received	2 doses	6	7.69%
	3 doses	72	92.31%





r: Pearson correlation coefficient

Figure 1: Age-dependant decrease in the amount of antibodies.