

Brief Original Article

## Serologic characteristics of hepatitis B virus among hill-tribe children in Omkoi district, Chiangmai province, Thailand

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### Abstract

**Introduction:** Thailand has integrated hepatitis B (HB) vaccination of newborns into the national Expanded Program on Immunization (EPI) in 1992. This has led to a dramatic decrease of HBsAg prevalence in children. However, HB vaccine coverage in remote areas is not well-known. This study aimed to investigate serologic characteristics of hepatitis B virus (HBV) among hill-tribe children in Omkoi District, Chiangmai Province, Thailand.

**Methodology:** This cross-sectional study was conducted on stored samples collected from hill-tribe children attending the primary/secondary school in Omkoi District in December 2014. Sera were tested for HBsAg, anti-HBs and anti-HBc using enzyme immunoassays (MUREX, DiaSorin, Italy). Samples with anti-HBc positive were further assessed for HBV DNA using an in-house HBV DNA semi-nested polymerase chain reaction (PCR) assay.

**Results:** Of 210 children evaluated, 4 (1.9%;95% CI 0.5-4.8) were HBsAg-positive. Of the 206 children HBsAg negative, 17 were anti-HBc and anti-HBs positive, 15 anti-HBc positive only, 26 anti-HBs positive only and 148 negative for both anti-HBc and anti-HBs. None of the children with anti-HBc were positive for HBV DNA.

**Conclusions:** A high percentage of children had no markers of HBV protection suggesting that HB vaccine coverage was not optimal in this area. Our results warrant HBV serologic investigations in other remote areas to assess whether HB vaccine coverage needs to be improved and to identify children who should be vaccinated.

**Key words:** hepatitis B virus; vaccine; serological markers; hill-tribe children; Thailand.

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### Introduction

Mother-to-child transmission has been the main route of hepatitis B virus (HBV) transmission in Asia [1]. In Thailand integration of hepatitis B (HB) vaccination of newborns into the national Expanded Program on Immunization (EPI) in 1992 has led to dramatic decrease of HBsAg prevalence in children [2,3]. HB vaccine coverage rate has been reported to be greater than 95% [4]; however, the coverage rate may differ between urban and rural areas [5], in particular in remote areas of the country. This study aimed to assess the serologic markers of HBV infection among hill-

tribe children in the remote district of Omkoi, Chiangmai Province, Thailand.

### Methodology

#### *Study population*

This cross-sectional study used stored frozen plasma samples that were collected in December 2014 during a survey study of anemia by Yanola *J et.al.* [6] among hill-tribe children attending the primary/secondary school in a rural area in the north region of Thailand, Omkoi District, Chiangmai province. All children present at the time of the survey were recruited. The study was reviewed and approved

by the Ethic Committee of Faculty of Associated Medical Sciences, Chiang Mai University (Approval number: AMSEC-61EM-003).

*Serological assays*

Plasma samples were tested for HBsAg using an enzyme immunoassay (MUREX HBsAg version 3, DiaSorin, Saluggia-Vercelli, Italy) (97% sensitivity and 98% specificity) and samples negative for HBsAg were then tested for anti-HBs and anti-HBc using ETI-AB-AUK-3 (99.1% sensitivity and 98.2% specificity) anti-HBs and MUREX anti-HBc (100% sensitivity and 99.7% specificity) according to the manufacturer’s protocols. Children with anti-HBs antibodies level > 10 international units/liter (IU/L) were considered as having protective immunity against HBV.

HBsAg-negative children were categorized into 4 groups according to their anti-HBs and anti-HBc serological results: 1) resolved HBV infection if anti-HBc and anti-HBs were positive, 2) exposed to HBV and had isolated anti-HBc, irrespective of whether they had recovered or actively infected, if anti-HBc positive only, 3) HB vaccinated if anti-HBs positive only, and 4) susceptible to HBV infection if both anti-HBc and anti-HBs were negative.

**Table 1.** Baseline characteristics of study population.

Categories	N (%) or Median (interquartile ranges)
<b>Gender (n = 210)</b>	
Male	77 (36.7%)
Female	133 (63.3%)
<b>Median age, year old (n = 209)</b>	
	11 (IQR: 10-13)
<b>Age, years (n = 209)</b>	
7	2 (1.0%)
8	18 (8.6%)
9	30 (14.3%)
10	37 (17.6%)
11	29 (13.8%)
12	26 (12.4%)
13	29 (13.8%)
14	38 (18.1%)
<b>Education Grade (n = 210)</b>	
Grade 2	3 (1.4%)
Grade 3	20 (9.5%)
Grade 4	31 (14.8%)
Grade 5	35 (16.7%)
Grade 6	35 (16.7%)
Grade 7	26 (12.4%)
Grade 8	27 (12.9%)
Grade 9	33 (15.7%)

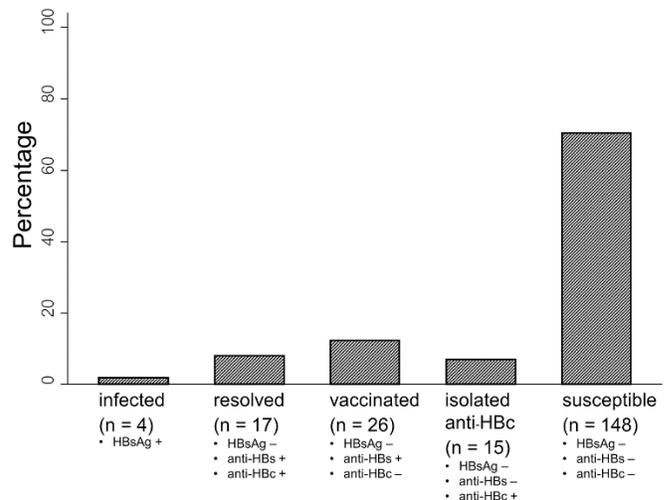
*Molecular assay*

Samples tested negative for HBsAg and positive for anti-HBc antibodies were tested for HBV DNA. HBV DNA was extracted using NucleoSpin Plasma XS (Macherey-Nagel, Dugen, Germany) following the manufacturer’s recommendations. DNA extracts were submitted to an in-house semi-nested polymerase chain reaction (PCR) (Lower limit of detection of 100 IU/mL). Briefly, in the first-round PCR, 10 µL of DNA extract was added into 40 µL of PCR master mix containing 1X Taq buffer with KCl, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.5 µM Primer A5-F (-66-GCT CCA GTT CAG GAA CAG TAA ACC C-90-), 0.5 µM Primer A2-R (-477-GGA CAA ACG GGC AAC ATA CCT TG-455-) and 0.02 U Taq DNA polymerase. First-round PCR conditions were an initial denaturation step of 94°C for 2 minutes, followed by 40 cycles of 1 minute at 94°C, 1 minute at 55°C and 1 minute at 72°C. For the second-round PCR, 10 µL of first-round PCR product (1,055 base pairs) were added into 40 µL of PCR master mix with the same conditions as in the first-round, with primer A2-R replaced by primer A3-R (-1121-AGA AAG GCC TTG TAA GTT GGC G-1100-) [7] and the annealing temperature was adjusted to 53°C. Second-round PCR product/Amplicons (411 base pairs) were visualized on a 2% agarose gel electrophoresis.

*Statistical analysis*

Characteristics of children including age at blood sampling, gender, educational grade are described using number and percentage and 95% confidence intervals (95%CI) for categorical data and median with interquartile range (IQR) for continuous data. Mann-

**Figure 1.** HBV serological statuses of hill-tribe children in Omkoi district.



Whitney U test was used to compare median of anti-HBs level between groups of HBsAg negative children. All analyses were performed using STATA version 14.1 software (Statacorp, Texas, USA). Differences were considered statistically significant if the p-value was  $\leq 0.05$ .

**Results**

*Baseline characteristics*

Samples of 210 children were included in the study. Of these 210 children, 77 (36.7%) were male and 133 (63.3%) were female. Their age ranged from 7-14 years with a median age of 11 years (IQR; 10-13). Their education grade ranged from grade 2 to 9 (Table 1).

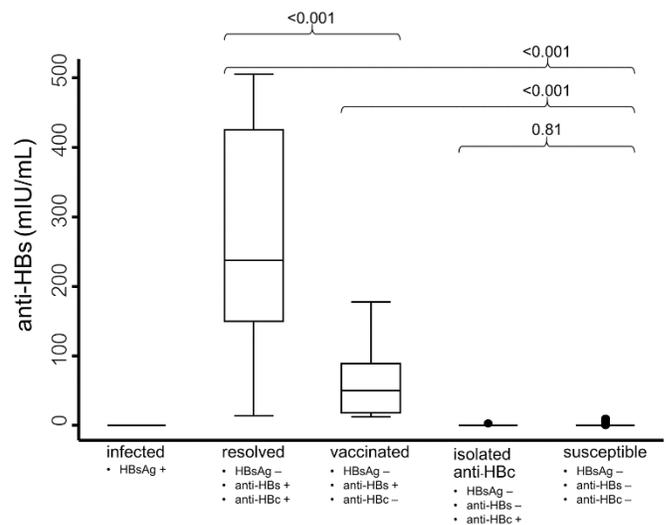
*Serological status of HBV infection*

Four children (1.9%;95% CI 0.5-4.8) were HBsAg-positive and all had HBV DNA detected. Two were males and 2 females with an age ranging between 11 and 14 years. Of 206 children HBsAg negative, 17 were anti-HBc and anti-HBs positive and were considered as having resolved their HBV infection, 15 were anti-HBc positive only and were considered as having been previously exposed to HBV with isolated anti-HBc, 26 anti-HBs positive only and considered as vaccinated and 148 anti-HBc and anti-HBs negative considered as susceptible to HBV infection (Figure 1). All children positive for anti-HBc, except one with insufficient sample, were subsequently tested for HBV DNA. None of them were positive for HBV DNA.

*Anti-HBs levels according to HBV serological status*

The median of anti-HBs levels was significantly higher in children who resolved from natural HBV

**Figure 2.** Anti-HBs antibody levels according to HBV serological statuses.

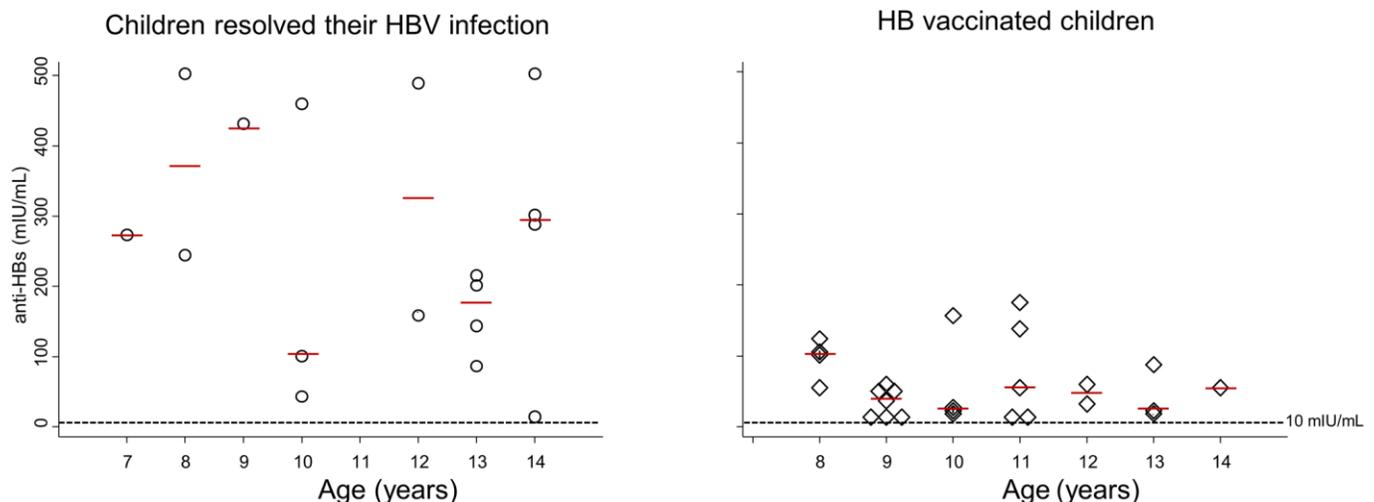


infection than in vaccinated children (238 mIU/mL, IQR: 149-425 versus 50 mIU/mL, IQR: 18-89,  $p < 0.001$ , Figure 2). This was also observed across all age groups (Figure 3).

**Discussion**

In this study of serologic HBV markers among hill-tribe children aged 7-14 years, we found a low proportion (12.4% positive for anti-HBs antibodies only) of children with immune protection against HBV and a high proportion (70.5%) of children with no HBV markers. Furthermore, there was a high rate (17.1%) of natural HBV infection: 1.9% of active infection, 8.1% of resolved HBV infection and 7.1% of isolated anti-

**Figure 3.** Levels of anti-HBs among children with resolved HBV infection versus HB vaccinated children according to their age. The bars indicate the averages of anti-HBs level in each group.



HBc status. Overall, our results indicate a low HB vaccine coverage in that population of children.

Since 2011, the EPI has been managed by the National Health Security Office and vaccine has been distributed by the Government Pharmaceutical Organization to public hospitals. The vaccines are administered free of charge. The coverage rate has increased from 15% in 1992 to 95% in 2000 and has reached levels of 99% in 2013 [8]. However, coverage has been shown to be higher among urban school children than among rural school children [5]. Our study shows a low proportion (12.4%) of vaccinated children and a high proportion (70.5%) of children with no HBV markers. All children were born after the year 2000 when the newborn HB vaccine was already integrated in the EPI. Thus, our results indicate that a large proportion of those children had not received HB vaccine as recommended in the national guidelines in Thailand. The low coverage of HB vaccine in hill-tribe school children may be due to the difficulties to access the health care facilities, the lack of knowledge of pregnant women about the need for antenatal care and the lack of information on HBV infection and prevention. A strategy to catch-up those unvaccinated children would be to implement “outreach care services”. Testing with HBsAg and anti-HBs antibody rapid tests will identify susceptible cases who could be vaccinated immediately at their village. HB vaccine can be transported and stored at room temperature for up to 1-3 months without losing its activity [9]. This strategy will be more effective with the help of Village Health Volunteers who usually provide general healthcare and health information to people in communities.

Furthermore, the high rate of natural HBV infection (1.9% of active infection and 15.2% of resolved HBV infection including those with isolated anti-HBc status) underlines the low HB vaccine coverage in that population of children. The prevalence of HBsAg positive was consistent with the 1.2% (8 of 680) prevalence reported in a study among schoolchildren aged 4-9 years in Chiangmai conducted during 1998-2000 [5]. The large cross-sectional study conducted in 2014 in 7 provinces of Thailand also showed that less than 2% of children under 20 years of age were HBsAg seropositive after 22 years’ implementation of the EPI [10]. We found 15% of children with positive anti-HBc, indicating that these children may have not been vaccinated although they were born after implementation of universal newborn HB vaccination and may have acquired HBV infection either perinatally from their mothers or horizontally during childhood from HBV-infected children or household contacts. We

found that none of the children with positive anti-HBc had detectable HBV DNA. However, the lower limit of detection of our technique was 100 IU/mL. We may have missed very low levels of HBV DNA and thus have underestimated the prevalence of HBV occult infection.

## Conclusions

Despite HB vaccine is free of charge for all newborns in Thailand, 17% of children in Omkoi district have been infected by HBV and a large proportion were still susceptible to HBV infection. Our results warrant HBV serologic investigations in other remote areas to assess whether HB vaccine coverage needs to be improved and to identify children who should be vaccinated.

## Acknowledgements

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**Conflict of interests:** No conflict of interests is declared.