Seroprevalence and factors associated with hepatitis B virus infection among the hill tribe youths, northern Thailand

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Abstract

Background: Hepatitis B virus (HBV) infection is a major viral infection, particularly in people living in the Western Pacific region, including the hill tribe people living in northern Thailand. This study aimed to estimate the prevalence of HBV infection and to detect the factors associated with HBV infection among hill tribe youths in Thailand.

Methods: A cross-sectional study was conducted to estimate the prevalence and determine the factors associated with HBV infection among hill tribe youths living in northern Thailand. A validated questionnaire and 5 mL blood sample were used for data collection. The Wondfo Diagnostic Kit®, the Wondfo One Step HBsAg Serum/Plasma Test®, and the Wondfo One Step HBsAg Serum/Plasma Test® were used for anti-HBsAg, HBsAg, and total anti-HBc detections, respectively. Logistic regression was used to detect associations between variables with an $\alpha = 0.05$ significance level.

Results: A total of 836 participants were included in the study; 62.7% were female, 58.9% were aged 15–17 years, 58.7% were Buddhist, 78.4% graduated high school, and 89.1% had no income. The majority were Akha (30.0%), Yao (16.3%), and Hmong (15.8%); 13.2% smoked, 21.5% used alcohol, 13.3% had tattoos, 3.9% experienced drug injection from illegal practitioners, and 35.7% had no history of HBV immunization. The prevalence of HBsAg was 3.0%; anti-HBs, 10.2%; and total anti-HBc, 8.1%. In the multivariate analysis, four variables were found to be significantly associated with HBV infection among the hill tribe youths: age, tribe, work experience, and number of partners. Those aged 18–20 years and 21–24 years had 2.13 times (95%CI = 1.35–3.29) and 2.39 times (95%CI = 1.05–3.90) greater odds of HBV infection, respectively, than those aged 15–17 years. Akha, Lahu, and Hmong youths had 3.12 times (95%CI = 1.07–9.12), 3.71 times (95%CI = 1.21–11.41), and 3.84 times (95%CI = 1.26–11.69) greater odds of HBV infection, respectively, than Lisu youths. Those who had experience working outside of the village had a 1.77 times (95%CI = 1.18–2.98) greater chance of HBV infection than those who did not have experience working outside of the village, and those who had ≥ 2 partners had a 2.66 times (95%CI = 1.96–3.87) greater chance of HBV infection than those who had no partner.

Conclusions: Effective HBV prevention programs should be promoted in Akha, Lahu, and Hmong youth populations, particularly to those who have sexual partners, work outside of the village and are aged 18–24 years.

Keywords: Hepatitis B, Seroprevalence, Hill tribe, Youths, HBsAg, Anti-HBs, Total anti-HBc
Background
Hepatitis B infection is an infectious diseases with one of the largest impacts on human health, with approximately 257 million infected people worldwide [1]. The target organ of the infection is the human liver [2]. The infection leads to several health problems, including acute and chronic diseases such as hepatitis, cirrhosis, and hepatocellular carcinoma (HCC), etc. [1]. Approximately 80–90% of infections occur in the first year of life, and 30–50% of infections that occur before the age of 6 progress to the chronic stage [3]. The chronic stage of HBV infection eventually leads to the development of HCC during middle age [4, 5]. HCC is one of the most invasive and aggressive cancers [6]. Moreover, HCC rates impact the national health system and health economics of a country.

According to a World Health Organization (WHO) report, people living in the Western Pacific region are the most vulnerable to HBV infection [1], with a prevalence rate of 6.2%. Regarding impacted persons, one-third of hepatitis B cases are reported from the Western Pacific region [7]. China has been recognized as one of the countries with the highest HBV burden, with a reported prevalence of 5.58% [8]. There are several minority populations living in South China [9]. Many of the so-called hill tribe people have been migrating and settling in northern Thailand since the nineteenth century [10]. Today, there are more than three million hill tribe people living in Thailand, which are classified into six main groups: Akha, Lahu, Hmong, Yao, Karen, and Lisu [11]. They have their own culture and lifestyles, including traditional practices that might be related to HBV infection, such as ear piercing and traditional acupuncture [12, 13]. In 2018, approximately 200,000–300,000 hill tribe people lived in Chiang Rai Province, which is located in the northernmost part of Thailand [11]. Today, the hill tribe people still maintain their own daily lifestyles and traditional practices; however, many villagers, particularly those who are living in Chiang Rai Province, are exposed to several risk factors outside their village through globalization. Chiang Rai Province borders Myanmar in the west, China in the north, and the Republic of Laos in the East.

The hill tribes in Thailand have become vulnerable to HBV infection for various reasons, such as their own traditional practices [11], low socioeconomic status [13], poor access to health care, language barriers [14], distance to health care settings [13], and stigmatization from health care providers [13]. In 2018, there were almost 3.5 million hill tribe people living in Thailand; however, some of them were not registered as Thai citizens [11]. Citizenship allows any individual to obtain a national identification card when they reach the age of 7 under Thai regulation [15]. The ID card is used to access all public services, including health care services and school attendance, free of charge.

Due to their living environment, social context, and socioeconomic status, the hill tribe populations in Thailand are at risk of HBV infection, particularly youths who belong to the third or fourth generations of the hill tribes living in Thailand today. The hill tribe youths are at a stage in their lives where it is very possible to be exposed to people outside their villages during different daily activities. Many hill tribe youths are exposed to people outside their villages due to work or attending school in a large city. Exposure to people and experiences outside the village, including sexual partners, tattoos, alcohol and illegal substances, have made them a new vulnerable population for HBV infection. There is little scientific information available regarding hepatitis B infection among hill tribe youths who are sexually active, eager to explore, and exposed to new experiences outside of their villages. Thus, the study aimed to estimate the prevalence and to determine the factors associated with HBV infection among hill tribe youths in northern Thailand.

Methods
Study design
A cross-sectional study was performed collect data from hill tribe youths.

Study setting
The participants were recruited from 60 selected hill tribe villages. Five villages of each tribe were selected by a simple random method from lists of the hill tribe villages located in Chiang Rai Province, northern Thailand. In 2016, there were 749 hill tribe villages in Chiang Rai, which included 316 Lahu villages, 243 Akha villages, 63 Yao villages, 56 Hmong villages, 36 Karen villages, and 35 Lisu villages. In 2016, a total of 41,366 hill tribe families lived in Chiang Rai Province [16].

Study population
The study population comprised hill tribe youths aged 15–24 years.

Eligible population
Eligible populations were hill tribe youths from one of the six hill tribes living in Chiang Rai Province.

Inclusion and exclusion criteria
The inclusion criteria were as follows: a) the participant self-identified as a member of one of the six hill tribes, b) the participant was aged between 15 and 24 years at the date of data collection, and c) the participant was fluent in Thai. However, selected participants who had a physical or mental condition that led to the inability to
provide essential information regarding the study protocols were excluded from the study.

Sample size
The sample size was calculated based on a formula for calculating the sample size in a cross-sectional study [17];

\[ n = \left[ \frac{Z^2 \alpha^2 PQ}{e^2} \right] \]

where \( n \) = sample size required, \( Z = 1.96 \), \( P = 0.10 \) [18], \( Q = 0.90 \) and \( e = 0.05 \). An average of 138 cases per tribe was required. Based on six tribes, a total of 829 participants were required for the analysis.

Research instruments
A questionnaire and 5 mL blood specimens were used as research instruments. A questionnaire was developed from a literature review and consultations with experts in the field. The questionnaire consisted of 31 questions categorized into three sections. In the first section, 10 questions were used to collect general information from the participants, such as sex, age, tribe, and marital status. In the second section, 8 questions were used to collect information on risk behaviors such as smoking, alcohol drinking, tattoos, and ear piercing. In the last section, 13 questions were used to collect information on the sexual behaviors of the participants, such as the number of partners, use of condoms during sexual intercourse, and sexual orientation.

Research instrument development
The questionnaire was tested for validity by the item-objective congruence technique (IOC), which was performed by three external experts in the relevant fields, a virologist, an infectious disease specialist, and an infectious epidemiologist. Questions with a score of less than 0.50 were excluded from the questionnaire, questions with a score between 0.50–0.70 were revised before use, and questions with a score of > 0.70 were used without modification.

Afterward, the questionnaire was piloted on 20 persons (10 males and 10 females) in the Mae Chan district of Chiang Rai Province who were similar to the study participants, with the aim of assessing the reliability and feasibility of the questionnaire.

Process of data gathering
After identifying all sixty selected hill tribe villages, village headmen were contacted 2 days before data collection. Access to villages was granted by district government officers. The lists of the target subjects were obtained from the village headmen. In each village, all eligible subjects were informed about the research objectives and protocols by the village headman. The study samples were randomly selected from the lists provided. All selected samples were appointed in the village. Upon reaching the village, all selected samples were provided all essential information and written informed consent was obtained before completing the questionnaire. Five milliliter blood samples were voluntarily collected by a professionally licensed medical technologist. Blood samples were kept in a proper container with cold packs and transferred to the laboratory on the same day.

Laboratory method
Laboratory tests were performed at the Mae Fah Luang Medical Laboratory. A rapid immunochromatographic method was used to detect anti-HBs and HBsAg markers. The Wondfo Diagnostic Kit was used to detect ant-HBsAg with 97.3% sensitivity and 99.2% specificity. The Wondfo One Step HBsAg Serum/Plasma Test was used to detect HBsAg with 96.2% sensitivity and 99.3% specificity. For the anti-HBc, the Wondfo One Step HBsAg Serum/Plasma Test was used with 93.0% sensitivity and 99.0% specificity.

Laboratory interpretation
Participants who were negative for HBsAg, total anti-HBc, and anti-HBs were susceptible to HBV infection. Participants who were negative for both HBsAg and total anti-HBc but positive for anti-HBs were defined as having immunity due to hepatitis B vaccination. Participants who were negative for HBsAg but positive for total anti-HBc and anti-HBs were defined as having immunity due to natural infection. HBV infection was defined as people who were positive for HBsAg and/or total anti-HBc after excluding individuals who had a serological profile of previous vaccination against HBV [19, 20].

Statistical analysis
All questionnaires and laboratory information were coded and double entered into an Excel sheet. Data were checked for errors, including missing values, before analysis. Data were analyzed by SPSS version 24, 2016 (SPSS, Chicago, IL). Descriptive and inferential statistics were used for analysis; general characteristics of participants were described by means, standard deviations, and percentages. Logistic regression was used to detect the associations between independent variables (general characteristics, risk behaviors, and sexual behaviors) and the dependent variable, HBV infection, at the \( \alpha = 0.05 \) significance level. The “ENTER” method was chosen in the logistic regression model, and statistically nonsignificant variables were excluded from the model; therefore, only statistically significant variables were included in the interpretation.
Results
A total of 836 participants were recruited for the study, and nobody refused to participate. The majority of participants were females aged 15–17 years (mean = 17.5, SD = 2.4) and of the Akha tribe. Most participants were single, in school, had no income, and had 4–6 family members. Half of the participants were Buddhist (Table 1).

Regarding the risk behaviors, different kinds of drug use were detected among the participants: 13.2% smoked, 21.5% drank alcohol, 2.3% used methamphetamines, and 4.3% used marijuana. Two-thirds experienced ear piercing, and 13.3% were tattooed. Some participants reported blood transfusions (3.9%), organ transplants (1.0%), a history of drug injection from illegal practitioners (3.9%), and acupuncture (1.8%). Only 8.5% were immunized for HBV, and 2.9% had at least one family member who had hepatitis. One-third of the participants reported that they had a sexual experience, 44.9% did not use a condom during their first sexual intercourse, and 11.5% had ≥2 partners (Table 2).

The prevalence rate of HBsAg positivity was 3.0%, anti-HBs positivity was 10.2%, total anti-HBC positivity was 8.1%, and both anti-HBs and anti-HBC positivity was 0.92%. The rate of negativity for anti-HBs but positivity for total anti-HBC was 7.4%. The prevalence rate of HBV infection was 10.3% (Table 3).

In the univariate analysis, five variables were associated with HBV infection among the hill tribe youths: age, tribe, marital status, work experience outside the village, and number of sexual partners. However, in the multivariate analysis, four variables remained associated with HBV infection. Those aged 18–20 years and 21–24 years had 2.13 times (95%CI = 1.35–3.29) and 2.39 times (95%CI = 1.05–5.90) greater odds of HBV infection, respectively, than those aged 15–17 years. Akha, Lahu, and Hmong youths had 3.12 times (95%CI = 1.07–9.12), 3.71 times (95%CI = 1.21–11.41), and 3.84 times (95%CI = 1.26–11.69) greater odds of HBV infection, respectively, than Lisu youths. Those who had experience working outside the village had a 1.77 times (95%CI = 1.18–2.98) greater chance of HBV infection than those who did not work outside the village, and those who had ≥2 sexual partners had a 2.66 times (95%CI = 1.96–3.87) greater chance of HBV infection than those who did not have a sexual partner (Table 4).

Discussion
In this study, the prevalence of HBsAg was 3.0%, the prevalence of anti-HBV was 10.2%, and the prevalence of HBV infection was 10.3%. The hill tribe youths in Thailand who were of the Akha, Lahu, and Hmong tribes; were older; had more than one partner; and had

| Table 1 General characteristics of the participants |
|--------------------------------------------------|
| Characteristics | n (%)       |
| Total          | 836 (100.0%) |
| Sex            |             |
| Male           | 312 (37.3)  |
| Female         | 524 (62.7)  |
| Age (years)    |             |
| 15–17          | 492 (58.9)  |
| 18–20          | 248 (29.7)  |
| 21–24          | 96 (11.5)   |
| Tribe          |             |
| Akha           | 251 (30.0)  |
| Lahu           | 110 (13.2)  |
| Hmong          | 132 (15.8)  |
| Yao            | 136 (16.3)  |
| Karen          | 130 (15.5)  |
| Lisu           | 77 (9.2)    |
| Marital status |             |
| Single         | 791 (94.6)  |
| Other          | 45 (5.4)    |
| Family members (persons) | |
| ≤ 3            | 92 (11.0)   |
| 4–6            | 529 (63.3)  |
| 7–10           | 181 (21.7)  |
| ≥ 11           | 34 (4.1)    |
| Religion       |             |
| Buddhism       | 491 (58.7)  |
| Christianity   | 339 (40.6)  |
| Islam          | 6 (0.7)     |
| Education      |             |
| Illiterate     | 21 (2.5)    |
| Primary school | 31 (3.7)    |
| High school    | 655 (78.4)  |
| Vocational school | 129 (15.4) |
| Occupation     |             |
| Unemployed     | 62 (7.4)    |
| Student        | 685 (81.9)  |
| Farmer         | 22 (2.6)    |
| Laborer        | 67 (8.1)    |
| Income         |             |
| Yes            | 91 (10.9)   |
| No             | 745 (89.1)  |
| Living place   |             |
| Own house      | 590 (70.6)  |
| Dormitory      | 208 (24.9)  |
| Other          | 38 (4.5)    |
experienced working outside the village had a higher risk of HBV infection.

Thailand has included the HBV vaccine in their expanded program of immunization (EPI) since 1992 [21–23]. All children are immunized with at least three doses of the HBV vaccine, with the first dose given 24 h after birth. In 2017, after 25 years of HBV vaccination through the EPI program, a 99.0% HBV vaccine coverage was reported for the whole country [22]. However, in our study, only 71 out of 836 participants (8.5%) confirmed having received HBV vaccination; 31.2% reported that they were not sure if they had been immunized, and 24.6% reported that they did not know their HBV immunization status. However, there were only 3 cases positive for HBsAg and 3 cases positive for anti-HBs among those who had a history of HBV immunization. These data differ from the 90–95% efficacy rate of the HBV vaccine after 3 standard doses declared by the

| Characteristics                  | n   | %  |
|----------------------------------|-----|----|
| Smoker                           |     |    |
| No                               | 726 | 86.8|
| Yes                              | 110 | 13.2|
| Alcohol use                      |     |    |
| No                               | 656 | 78.5|
| Yes                              | 180 | 21.5|
| Methamphetamine use             |     |    |
| No                               | 817 | 97.7|
| Yes                              | 19  | 2.3 |
| Heroin use                       |     |    |
| No                               | 823 | 98.4|
| Yes                              | 13  | 1.6 |
| Crystal methamphetamine use     |     |    |
| No                               | 825 | 98.7|
| Yes                              | 11  | 1.3 |
| Opium use                        |     |    |
| No                               | 824 | 98.6|
| Yes                              | 12  | 1.4 |
| Marijuana use                    |     |    |
| No                               | 800 | 95.7|
| Yes                              | 36  | 4.3 |
| Tattooed                         |     |    |
| No                               | 725 | 86.7|
| Yes                              | 111 | 13.3|
| Ear piercing                     |     |    |
| No                               | 302 | 36.1|
| Yes                              | 534 | 63.9|
| History of blood transfusion     |     |    |
| No                               | 803 | 96.1|
| Yes                              | 33  | 3.9 |
| History of organ transplant      |     |    |
| No                               | 828 | 99.0|
| Yes                              | 8   | 1.0 |
| History of medical surgery       |     |    |
| No                               | 733 | 87.7|
| Yes                              | 103 | 12.3|
| Injection from illegal practitioners |   |    |
| No                               | 803 | 96.1|
| Yes                              | 33  | 3.9 |
| Acupuncture                      |     |    |
| No                               | 821 | 98.2|
| Yes                              | 15  | 1.8 |
| Work experience outside the village |   |    |
| No                               | 693 | 82.9|
| Yes                              | 143 | 17.1|

| Characteristics                  | n   | %  |
|----------------------------------|-----|----|
| Work experience abroad           |     |    |
| No                               | 826 | 98.8|
| Yes                              | 10  | 1.2 |
| Used a shared toothbrush         |     |    |
| No                               | 656 | 78.5|
| Yes                              | 180 | 21.5|
| History of hepatitis B vaccination |   |    |
| Yes                              | 71  | 8.5 |
| No                               | 298 | 35.7|
| Not sure                         | 261 | 31.2|
| Unknown                          | 206 | 24.6|
| Family member history of hepatitis |   |    |
| No                               | 481 | 57.5|
| Yes                              | 24  | 2.9 |
| Not sure                         | 120 | 14.4|
| Unknown                          | 211 | 25.2|
| Sexual experience                |     |    |
| No                               | 593 | 70.9|
| Yes                              | 243 | 29.1|
| Used a condom during first sexual intercourse |   |    |
| No                               | 109 | 44.9|
| Yes                              | 120 | 49.4|
| Unrembered                       | 14  | 5.7 |
| Number of partners (persons)     |     |    |
| None                             | 593 | 70.9|
| 1                                | 147 | 17.6|
| ≥ 2                              | 96  | 11.5|

Table 2 Risk behaviors among the participants

Table 2 Risk behaviors among the participants (Continued)
Ministry of Public Health in Thailand [24]. This reflects the level of access to health care services among the hill tribe people in Thailand, which was reportedly low due to factors such as distance, language and stigmatization [14, 25]. There are also many factors influencing the efficacy of HBV immunization, such as nutritional status [26], route of vaccine delivery [27], age at vaccination, underlying diseases [28, 29], and host genetics [30].

Moreover, with low parental education rates and low health literacy, including the administration of the HBV immunization in children while they are young, may have led to missing responses to question in the study which might impact the analysis. During the data collection, many sources of information were investigated regarding the HBV immunization status of a participant. A study conducted at the Thai-Myanmar border reported that administering HBV vaccines to people living in the border area was highly effective in preventing the disease [31].

Regarding HBsAg seroprevalence, countries in the Western Pacific region had a rate of 6.1% [1]. In Taiwan, the seroprevalence of anti-HBsAg and total anti-HBc was 8.1% and increased according to age, particularly in those aged 18–25 years [32]. In 2015, Leroy et al. [17] reported that the overall seroprevalence of HBsAg in the Thai general population was 0.6–3.1%. Lana, et al. [21] reported that the HBs-carrier rate of Thai people aged 11–20 was 0.69%. These findings are lower than the seroprevalence detected in our study among hill tribe youths in Thailand.

Moreover, Banks, et al. [33] reported that the hepatitis B seroprevalence among pregnant women who lived on the Thailand-Myanmar border was 8.3%. Another study that was conducted with children and adolescents with HIV infection in six countries in Southeast Asia, including Thailand, reported that the prevalence of HBsAg was 4.7% [34]. Pichainarong, et al. [35] reported that the hepatitis B seroprevalence among married hill tribe women in northern Thailand was 8.2%. These studies presented HBsAg seroprevalence rates close to the rates in our study due to similar population demographics. Another reason might be an increase in the number of people who are exposed to people outside their village (17.1%). Today, many hill tribe people prefer to work outside of their village to earn money to support their family [36]. In fact, hill tribe people, particularly those aged below 25 years, should have low HBsAg since they were born after the implementation of HBV vaccination in Thailand. Another reason for high anti-HBsAg and anti-HBc levels among hill tribe people is due to low seroclearance, particularly in those who were infected at an older age [37, 38].

Regarding age and HBV infection, Taleban et al. [39] reported that increased age was one of the most significant factors for HBV infection in Iran. A study among health care professionals in Ethiopia in 2017 also reported that people who were older in age had a significantly greater risk of HBV infection than those younger in age [40]. Another study conducted among prisoners in Iran reported that age was the significant factor associated with HBV infection [41]. These findings support our study, which found that the older age group had a greater risk of HBV infection among the hill tribe youths in northern Thailand, even though they were born after the national implementation of HBV vaccination in Thailand.

Slovakia reported that some group of people had a greater risk than others; the Roma population had a greater risk of HBV infection than both the non-Roma population and those with tattoos [42]. Our study also found that some tribes had a greater risk of HBV infection than others.

The number of partners was highly associated with HBV infection in pregnant women in Ethiopia [43] and Tanzania [44]. A study among female workers in Laos also reported that the number of partners was associated with

Table 3 Prevalence of HBsAg and anti-HBs

| Characteristics                      | n | %  |
|--------------------------------------|---|----|
| Total                                | 836 | 100.0 |
| HBsAg                                |    |    |
| Negative                             | 811 | 97.0 |
| Positive                             | 25  | 3.0 |
| Received HBV vaccination             | 3   | 12.0 |
| No history of HBV vaccination        | 6   | 24.0 |
| Not sure                             | 9   | 36.0 |
| Unknown                              | 7   | 28.0 |
| Anti-HBs                             |    |    |
| Negative                             | 751 | 89.8 |
| Positive                             | 85  | 10.2 |
| Received HBV vaccination             | 3   | 3.5 |
| No history of HBV vaccination        | 32  | 37.6 |
| Not sure                             | 31  | 36.5 |
| Unknown                              | 19  | 22.4 |
| Total anti-HBc                       |    |    |
| Negative                             | 768 | 91.9 |
| Positive                             | 68  | 8.1 |
| Received HBV vaccination             | 0   | 0.0 |
| No history of HBV vaccination        | 12  | 17.6 |
| Not sure                             | 27  | 39.8 |
| Unknown                              | 29  | 42.6 |
| Anti-HBs-positive and anti-HBc-positive | 4  | 0.9 |
| Anti-HBs-negative and anti-HBc-positive | 64 | 7.4 |
| HBV infection                        | 86  | 10.3 |
## Table 4: Univariate and multivariate analyses of factors associated with HBV infection

| Factors                  | n     | %   | OR   | 95%CI   | p-value | OR_{Adj} | 95%CI   | p-value |
|--------------------------|-------|-----|------|---------|---------|----------|---------|---------|
| Sex                      |       |     |      |         |         |          |         |         |
| Male                     | 312   | 37.3| 1    |         |         |          |         |         |
| Female                   | 524   | 62.7| 0.70 | 0.47–1.06| 0.707   |          |         |         |
| Age (years)              |       |     |      |         |         |          |         |         |
| 15–17                    | 492   | 58.9| 1    |         |         |          |         |         |
| 18–20                    | 248   | 29.7| 2.14 | 1.38–3.34| 0.001a | 2.13     | 1.35–3.29| 0.001a |
| 21–24                    | 96    | 11.4| 2.39 | 1.33–4.30| 0.004a | 2.39     | 1.05–3.90| 0.005a |
| Tribe                    |       |     |      |         |         |          |         |         |
| Akha                     | 251   | 30.0| 3.05 | 1.05–8.87| 0.040a | 3.12     | 1.07–9.12| 0.037a |
| Lahu                     | 110   | 13.2| 4.30 | 1.41–13.10| 0.010a | 3.71     | 1.21–11.41| 0.022a |
| Hmong                    | 132   | 15.8| 3.65 | 1.20–11.02| 0.022a | 3.84     | 1.26–11.69| 0.018a |
| Yao                      | 136   | 16.3| 2.09 | 0.66–6.60| 0.207   | 2.46     | 0.77–7.87| 0.127   |
| Karen                    | 130   | 15.5| 2.02 | 0.63–6.45| 0.232   | 1.86     | 0.58–5.98| 0.293   |
| Lisu                     | 77    | 9.2 | 1    |         |         |          |         |         |
| Marital status           |       |     |      |         |         |          |         |         |
| Single                   | 791   | 94.6| 1    |         |         |          |         |         |
| Other                    | 45    | 5.4 | 2.26 | 1.11–4.60| 0.025a |          |         |         |
| Number of family members (persons) | | | | | | | | |
| 1–3                      | 92    | 11.0| 1    |         |         |          |         |         |
| 4–6                      | 529   | 63.3| 1.40 | 0.67–2.92| 0.361   |          |         |         |
| 7–10                     | 181   | 21.7| 1.47 | 0.65–3.31| 0.343   |          |         |         |
| ≥11                      | 34    | 4.1 | 1.97 | 0.64–6.04| 0.233   |          |         |         |
| Religion                 |       |     |      |         |         |          |         |         |
| Buddhism                 | 491   | 58.7| 1    |         |         |          |         |         |
| Christianity             | 339   | 40.6| 0.81 | 0.53–1.24| 0.345   |          |         |         |
| Islam                    | 6     | 0.7 | 1.22 | 0.14–10.62| 0.855   |          |         |         |
| Education                |       |     |      |         |         |          |         |         |
| Illiterate               | 21    | 2.5 | 1    |         |         |          |         |         |
| Primary school           | 31    | 3.7 | 2.77 | 0.51–14.90| 0.235   |          |         |         |
| High school              | 655   | 78.4| 1.36 | 0.31–5.94| 0.651   |          |         |         |
| Vocational school        | 129   | 15.4| 1.64 | 0.35–7.62| 0.527   |          |         |         |
| Occupation               |       |     |      |         |         |          |         |         |
| Unemployed               | 62    | 7.4 | 1    |         |         |          |         |         |
| Student                  | 685   | 81.9| 0.83 | 0.39–1.75| 0.632   |          |         |         |
| Farmer                   | 22    | 2.6 | 2.20 | 0.68–7.14| 0.186   |          |         |         |
| Labor                    | 67    | 8.1 | 1.03 | 0.39–2.73| 0.948   |          |         |         |
| Income                   |       |     |      |         |         |          |         |         |
| No                       | 745   | 89.1| 1    |         |         |          |         |         |
| Yes                      | 91    | 10.9| 1.08 | 0.52–1.90| 0.993   |          |         |         |
| Smoker                   |       |     |      |         |         |          |         |         |
| No                       | 726   | 86.8| 1    |         |         |          |         |         |
| Yes                      | 110   | 13.2| 1.34 | 0.77–2.33| 0.287   |          |         |         |
| Alcohol use              |       |     |      |         |         |          |         |         |
| No                       | 656   | 78.5| 1    |         |         |          |         |         |
| Factors                              | n   | %    | OR   | 95%CI     | p-value | OR Adj | 95%CI     | p-value |
|-------------------------------------|-----|------|------|-----------|---------|--------|-----------|---------|
| Methamphetamine use                 |     |      |      |           |         |        |           |         |
| No                                  | 817 | 97.7 | 1    |           |         |        |           |         |
| Yes                                 | 19  | 2.3  | 1.24 | 0.35–4.34 | 0.732   |        |           |         |
| Heroin use                          |     |      |      |           |         |        |           |         |
| No                                  | 823 | 98.4 | 1    |           |         |        |           |         |
| Yes                                 | 13  | 1.6  | 1.20 | 0.26–5.50 | 0.811   |        |           |         |
| Crystal methamphetamine use        |     |      |      |           |         |        |           |         |
| No                                  | 825 | 98.7 | 1    |           |         |        |           |         |
| Yes                                 | 11  | 1.3  | 0.65 | 0.08–5.18 | 0.690   |        |           |         |
| Tattooed                            |     |      |      |           |         |        |           |         |
| No                                  | 725 | 86.7 | 1    |           |         |        |           |         |
| Yes                                 | 111 | 13.3 | 1.13 | 0.63–2.00 | 0.674   |        |           |         |
| Ear piercing                        |     |      |      |           |         |        |           |         |
| No                                  | 302 | 36.1 | 1    |           |         |        |           |         |
| Yes                                 | 534 | 63.9 | 0.94 | 0.62–1.43 | 0.788   |        |           |         |
| History of blood transfusion        |     |      |      |           |         |        |           |         |
| No                                  | 803 | 96.1 | 1    |           |         |        |           |         |
| Yes                                 | 33  | 3.9  | 0.65 | 0.19–2.16 | 0.484   |        |           |         |
| History of medical surgery          |     |      |      |           |         |        |           |         |
| No                                  | 733 | 87.7 | 1    |           |         |        |           |         |
| Yes                                 | 103 | 12.3 | 1.04 | 0.57–1.90 | 0.889   |        |           |         |
| Injection from illegal practitioners|     |      |      |           |         |        |           |         |
| No                                  | 803 | 96.1 | 1    |           |         |        |           |         |
| Yes                                 | 33  | 3.9  | 0.65 | 0.19–2.16 | 0.484   |        |           |         |
| Acupuncture                         |     |      |      |           |         |        |           |         |
| No                                  | 821 | 98.2 | 1    |           |         |        |           |         |
| Yes                                 | 15  | 1.8  | 1.01 | 0.22–4.56 | 0.984   |        |           |         |
| Used a shared toothbrush            |     |      |      |           |         |        |           |         |
| No                                  | 656 | 78.5 | 1    |           |         |        |           |         |
| Yes                                 | 180 | 21.5 | 1.02 | 0.62–1.65 | 0.937   |        |           |         |
| History of hepatitis B vaccination  |     |      |      |           |         |        |           |         |
| Yes                                 | 71  | 8.5  | 1    |           |         |        |           |         |
| No                                  | 298 | 35.7 | 1.58 | 0.64–3.90 | 0.319   |        |           |         |
| Not sure                            | 261 | 31.2 | 1.96 | 0.79–4.83 | 0.143   |        |           |         |
| Unknown                             | 206 | 24.6 | 1.56 | 0.61–3.97 | 0.346   |        |           |         |
| Family history of hepatitis         |     |      |      |           |         |        |           |         |
| No                                  | 481 | 57.5 | 1    |           |         |        |           |         |
| Yes                                 | 24  | 2.9  | 0.58 | 0.13–2.53 | 0.471   |        |           |         |
| Not sure                            | 120 | 14.4 | 1.05 | 0.59–1.87 | 0.852   |        |           |         |
| Unknown                             | 211 | 25.2 | 0.89 | 0.55–1.46 | 0.670   |        |           |         |
| Work experience outside the village |     |      |      |           |         |        |           |         |
| No                                  | 693 | 82.9 | 1    |           |         |        |           |         |
| Yes                                 | 143 | 17.1 | 1.92 | 1.20–3.07 | 0.006a  | 1.77   | 1.18–2.98 | 0.004a  |
HBV infection [45]. Another study in Bangkok reported that the number of partners was associated with HBV infection [46]. These findings are similar to our study, which found that the number of partners was significantly associated with HBV infection among hill tribe youths, particularly those who had experience working outside of the village. Some studies [47, 48] conducted in hill tribe people in Thailand reported that working outside of the village was a factor associated with HIV and hepatitis B coinfection in northern Thailand.

Marital status was found to be associated with HBV infection in the univariate model; however, there was no statistical association with HBV infection in the multivariate model. A possible reason is for this result may be that many hill tribes have a monogamous culture, except the Akha who accept polygamy in their culture [25]. Those who have multiple partners often do so before marriage; once they are married, the opportunity for HBV infection is reduced. Several studies have also shown that marital status is not associated with HBV infection [49, 50].

Recall bias and misclassification were major limitations in the study. Recall bias [51, 52] happened since many questions regarding previous experiences were asked. For instance, a large proportion answered “not sure” or “unknown” when asked about their history of hepatitis B vaccination. These answers may have led to misclassification during analysis. However, the researchers were aware of these issues and repeated the questions during the interview to ensure the participants understood the questions before providing their answers.

All the HBsAg-positive participants were provided additional information regarding immunization at a registered hospital to protect against HBV infection.

**Conclusion**

The hill tribe youths in Thailand are at risk of hepatitis B infection, particularly those who work outside the village and have multiple sexual partners. Due to globalization, young children in the hill tribes should be immunized with the HBV vaccine through the EPI program of Thailand to ensure that they are immune to HBV infection. Moreover, suitable health education programs, such as safe sex practices, should be developed and implemented in Akha, Lahu, and Hmong youth populations aged ≥18 years.

**Abbreviations**

- Anti-HBc: Hepatitis core antibody
- Anti-HBs: Hepatitis B surface antibody
- CI: Confident interval
- EPI: Expanded program of immunization
- HBsAg: Hepatitis B surface antigen
- HBV: Hepatitis B virus
- HCC: Hepatocellular carcinoma
- IOC: Item-objective congruence technique
- MSM: Men who have sex with men
- WHO: World Health Organization

**Acknowledgements**
The author would like to thank all the participants for kindly providing essential information. I also would like to thank the National Research Council of Thailand, Mae Fah Luang University, and the Center of Excellence for the Hill tribe Health Research for grant support. Finally, we would like to express our special thank to colleagues from WHO-Thailand for technical support.

**Funding**
TA received funding from The National Research Council of Thailand and Mae Fah Luang University (Grant No. 60207020106). The funders had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

**Availability of data and materials**
Additional data could request at email: Tawatchai.api@mfu.ac.th

### Table 4

Univariate and multivariate analyses of factors associated with HBV infection (Continued)

| Factors                                    | n   | %   | OR   | 95% CI    | p-value | OR Adj | 95% CI    | p-value |
|--------------------------------------------|-----|-----|------|-----------|---------|--------|-----------|---------|
| Work experience abroad                     |     |     |      |           |         |        |           |         |
| No                                         | 826 | 98.8| 1    |           |         |        |           |         |
| Yes                                        | 10  | 1.2 | 2.88 | 0.73–11.30| 0.130   |        |           |         |
| Sexual experience                          |     |     |      |           |         |        |           |         |
| Yes                                        | 243 | 29.1| 1.10 | 0.71–1.71 | 0.648   |        |           |         |
| No                                         | 593 | 70.9| 1    |           |         |        |           |         |
| Used a condom during first sexual intercourse|   |     |      |           |         |        |           |         |
| Yes                                        | 120 | 49.4| 1    |           |         |        |           |         |
| No                                         | 109 | 44.9| 1.49 | 0.70–3.17 | 0.293   |        |           |         |
| Does not remember                         | 14  | 5.7 | 1.26 | 0.25–6.23 | 0.775   |        |           |         |
| Number of sexual partners (person)         |     |     |      |           |         |        |           |         |
| None                                       | 593 | 84.7| 1    |           |         |        |           |         |
| 1                                         | 67  | 9.6 | 0.43 | 0.15–1.22 | 0.113   | 0.57   | 0.28–1.19| 0.099   |
| ≥ 2                                        | 40  | 5.7 | 2.97 | 1.90–4.30| 0.033a  | 2.66   | 1.96–3.87| 0.047a  |

* Significant at α = 0.05
The authors declare that they have no competing interests.

Consent for publication

process. Those participants aged less than 18 years, parents were asked to sign Luang University Research Ethics Committee on Human Research (REH-60001).

Ethics approval and consent to participate

Program from Harvard University.

Humphrey (Fulbright) program and in the Global Health Delivery Intensive of Excellence for the Hill-tribe Health Research and trained in infectious disease Dr. Tawatchai Apidechkul is the Assistant Professor and Director of the Center.

TA planned and conducted the project, collected data, analyzed, drafted and proofread the final version of the manuscript. The author read and approved proofread the final version of the manuscript. The author read and approved

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 26 November 2018 Accepted: 28 January 2019

Published online: 06 February 2019

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