Seroprevalence and factors associated with hepatitis B infection among the hill tribe adult population in Thailand: a cross-sectional study

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Abstract

Background: Hepatitis B virus (HBV) infection is one of the greatest public health burdens, particularly for people living with several barriers to access to health care services, such as the hill tribe adult population in Thailand. People aged 25 years and over who are out of the target population for HBV immunization under the national Expanded Program on Immunization (EPI) are at risk of HBV infection. The study aimed to estimate the prevalence and determine the factors associated with HBV infection among hill tribe adults aged 25 years and over living in Chiang Rai Province, Thailand.

Methods: A cross-sectional study design was used to collect information on hill tribe adults aged 25 years and over living in 36 selected hill tribe villages in Chiang Rai Province. All people living in the selected villages who met the criteria were invited to participate in the study. A validated questionnaire and a 5-mL blood specimen were used as research instruments. Hepatitis B surface antigen (HBsAg), antibody to hepatitis B surface (anti-HBs), and antibody to hepatitis B core (anti-HBc) were detected by using the Wondfo Test Kit®, which has high sensitivity and specificity. Logistic regression was used to detect the associations between variables at the significance level of α = 0.05.

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Background

Hepatitis B virus infection is the greatest infectious disease in the human population, with approximately 257 million people living with chronic hepatitis B infection, which is defined by HBsAg positivity globally [1]. It is a common infectious disease that is transmitted person-to-person during delivery [2] and through contaminated blood [3] and other body fluids [2]. The targeted organ of the infection is the human liver [4]. Afterward, wide ranges of pathogenesis and complications could occur to adversely affect the infected liver’s health [5]. The serious final stage of infection is cancer, which mostly presents as aggressive progression for the organ with a very poor prognosis [6]. The WHO estimated that 887,000 deaths are reported every year from cirrhosis and hepatocellular carcinoma resulting from hepatitis B infection [1]. The WHO also reported that the highest prevalence of hepatitis B infection was in the Asia Pacific region (6.2%) [1]. A total of US$58.7 billion is needed to address hepatitis among 67 low- and middle-income countries by 2030, which could prevent 90.0% of new cases of infection and save the lives of 65.0% of those with existing cases of infection, including individuals in Thailand [7].

Thailand reported 2.2–3 million people who were hepatitis B carriers and who were HBsAg positive [8]. Thailand has included the hepatitis B vaccine on one of the lists in the Expended Program on Immunization (EPI) for almost 25 years since it was first implemented in 1992. Since then, this program has reduced the HBV carrier rate among children aged younger than 25 years by less than 1.0%. However, a high prevalence of those aged 25 years and older were still reported to be carriers of hepatitis B, with an average of 5.9% [9]. The hepatitis B vaccine has significantly reduced the number of hepatitis carriers and other medical expenses and has been used for treatment and care related to hepatitis B viral infection in the Thai population. However, this does not mean that all Thai people can access health care services equality, especially immunizations for children and other targeted populations under the national EPI, even if they do not need to pay a fee [10]. In addition, those who do not fall within the target of the Thai national EPI are requested to pay for US$70 for each HBV vaccine (three standard doses) [11]. Some Thai people live in poor economic conditions, have low levels of education and face several barriers to access to medical services, such as language, distance, and stigmatization [12]. The hill tribe people are a good example of those with atypical access to Thai medical services, since they are living in rural areas and have specific cultures and perceptions, particularly with respect to access to medical services, including immunization for children [13–15].

The hill tribe people migrated from southern China and settled down along the borders of Thailand-Myanmar-Laos over a couple centuries [16]. They are now composed of six main tribes: Akha, Lahu, Hmong, Yao, Karen, and Lisu [16]. In 2019, approximately 2.5–3.5 million people lived in Thailand [15], and 250,000–350,000 people lived in Chiang Rai Province [17]. Approximately 70.0% [18] of the hill tribe people have Thai identification cards (IDs), which are used for access to all public services, including health care services [19].

Unfortunately, the hill tribe people are living in the era of HBV vaccine implementation in Thailand, but the prevalence estimates of hepatitis B surface carrier (HBsAg) and the total hepatitis B infection were reported to be higher among the hill tribe people than in
the Thai population in the same age category at 10.3% [20]. With the specific history of those in the hill tribes who migrated from China, which has the highest prevalence of hepatitis B infection in the world [21], and given these individuals’ very limited access to health care services, the hill tribe population in Thailand is at risk of hepatitis B infection. Moreover, lifestyles and practices such as ear piercing [22], high alcohol consumption [23] and other substance use, including drug injection by untrained health professionals [24], all while living far away from the city, could lead individuals to become vulnerable to hepatitis B infection.

There is no scientific information regarding hepatitis B infection in the hill tribe population aged 25 years and older before the generation of the hepatitis B vaccine implementation in Thailand. Thus, the study aimed to estimate the prevalence of hepatitis B infection by detecting hepatitis B surface antigen (HBsAg), antibody to hepatitis B surface (anti-HBsAg), and total antibody to hepatitis B core (anti-HBc) according to the standard guidelines of the Centers for Disease Control and Prevention (CDC, US) [25].

Methods
Study design
A community-based cross-sectional study was used to collect information from the participants.

Study settings
The study was conducted in the hill tribe villages in Chiang Rai Province, Thailand. There are six main hill tribes living in the study settings: Akha, Lahu, Hmong, Yao, Karen, and Lisu. In 2019, 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 [17]. Six villages in each tribe were randomly selected to be study settings from the lists of the hill tribe villages in 2019 [17]; therefore, 36 hill tribe villages located in 14 districts in Chiang Rai Province served as the study settings [17].

Study population
The study population included hill tribe people who lived in the selected villages and met the eligibility criteria. The eligibility criteria for the study were as follows: i) belonging to one of the six main hill tribes in Thailand, ii) able to provide essential information according to the study protocol, and iii) aged 25 years and over.

Study sample and sample size calculation
The sample size was calculated from the standard formula of a cross-sectional study (Z = 1.96, \( P = 0.13 \) [20], \( Q = 0.87 \) and \( e = 0.05 \)); therefore, at least 246 participants in each tribe were required for the analysis. Thus, a minimum of 1475 participants were needed in the whole study.

Research instruments and their development
The questionnaire was developed from the literature review and was discussed with the medical staff working in an infectious diseases clinic at the Chiang Rai Regional Hospital (a tertiary hospital). The content validity of the questionnaire was also determined by the item-objective congruence (IOC) technique by three external experts (one epidemiologist, one public health specialist, and one infectious diseases physician), who provided their opinions regarding the relevance of the questions and the content of the study. The questions scoring less than 0.50 were excluded from the questionnaire set, those scoring between 0.51–0.70 were revised as comments obtained by the experts, and those scoring equal or more than 0.71 were included in the set of questionnaires.

Afterwards, the feasibility and ordering of the questions on the questionnaire were determined through its piloting in 15 samples with characteristics similar to those of the study participants at Mae Chan Hospital, Chiang Rai Province, Thailand. After the questionnaire was revised following the pilot test, the final questionnaire consisted of three parts. Part one included 10 questions that were used to collect the sociodemographic characteristics of the participants, such as age, sex, religion, marital status, occupation, and income. Part two included 14 questions that were used to collect information on risk behaviors related to hepatitis B infection, such as alcohol use, methamphetamine use, tattooing, history of blood transfusion, history of organ transplantation, and history of hepatitis B vaccination. Three open-ended questions were provided to collect laboratory results.

Data collection procedure
After approval was obtained for the research ethical consideration, development and completion of the questionnaire, all 14 relevant district government officers were contacted by the researchers to obtain their approval for access to the villages. Afterward, village headmen were contacted and were provided with details on the study. Targeted populations who met the criteria were informed regarding the execution of the study and made appointments for data collection 3 days prior.
On the date of data collection, all participants were asked to obtain the informed consent form after providing all essential information for the study, followed by 5-mL blood specimens being drawn by licensed health professionals. The questionnaire was completed by self-administration in a private and confidential room. Those who could not understand Thai received assistance in completing the questionnaire by village public health volunteers who were fluent in both Thai and local languages. All processes lasted for 30 min each.

**Laboratory methods**

Five-milliliter blood specimens were drawn from all participants. The laboratory analysis for hepatitis B markers HBsAg, anti-HBs, and total anti-HBc was performed at the Mae Fah Luang Central Medical Laboratory on the same day that blood specimens were drawn by licensed medical technicians.

The Wondfo One Step HBsAg Serum/Plasma® was used for the detection of HBsAg. It has 96.2% sensitivity and 99.3% specificity. The Wondfo Diagnostic Kit® was used for the detection of anti-HBs and total anti-HBc, with 97.3% sensitivity and 99.2% specificity.

**Outcomes**

Hepatitis B infection was defined based on the standard guideline for the interpretation of hepatitis B serologic test results of the Centers for Disease Control and Prevention (CDC), United States [25], such that those who were positive for either or both HBsAg and anti-HBs, except for those who had a negative anti-HBc, were considered to have hepatitis B infection.

**Statistical analysis**

Data were double-entered into an Excel sheet and checked for errors before being transferring into SPSS program version 24 (SPSS, Chicago, IL) for analysis. Continuous and categorical data were described properly to present the general characteristics of the participants and their behaviors, including hepatitis B biomarkers. Logistic regression was used to identify the factors associated with hepatitis B infection at the significance level of $\alpha = 0.05$. 

| Table 1 | General characteristics of participants (Continued) |
|---------|---------------------------------------------------|
| Characteristic | Number | Percent |
|----------|--------|---------|
| Total | 1491 | 100.0 |
| Sex | | |
| Male | 585 | 39.2 |
| Female | 906 | 60.8 |
| Age (years) | | |
| < 30 | 66 | 4.4 |
| 30–60 | 1212 | 81.3 |
| > 60 | 213 | 14.3 |
| Mean = 48.9 years, SD = 11.0, Min = 25, Max = 78 |
| Marital status | | |
| Single | 73 | 4.9 |
| Married | 1282 | 86.0 |
| Ever married | 136 | 9.1 |
| Education | | |
| Illiterate | 819 | 54.9 |
| Primary school | 383 | 25.7 |
| Secondary school | 217 | 14.6 |
| High school and above | 72 | 4.8 |
| Religion | | |
| Buddhist | 830 | 55.7 |
| Christian | 661 | 44.3 |
| Tribe | | |
| Akha | 295 | 19.8 |
| Lahu | 346 | 23.2 |
| Hmong | 299 | 20.1 |
| Yao | 167 | 11.2 |
| Lisu | 68 | 4.6 |
| Karen | 316 | 21.2 |
| Having Thai ID card | | |
| Yes | 1420 | 95.2 |
| No | 71 | 4.8 |
| Occupation | | |
| Farmer | 1302 | 87.3 |
| Working in a trade | 36 | 2.4 |
| Daily wage employee | 153 | 10.3 |
| Right to access free health care services | | |
| Thai universal health scheme | 1361 | 91.3 |
| State enterprise officer | 15 | 1.0 |
| Social security scheme | 40 | 2.7 |
| Private insurance | 4 | 0.3 |
| No | 71 | 4.8 |
| Number of family members (persons) | | |
| < 4 | 725 | 48.6 |
| 4–7 | 596 | 40.0 |
| Annual income (baht) | | |
| < 10,000 | 158 | 10.6 |
| 10,001–50,000 | 929 | 62.3 |
| > 50,000 | 404 | 27.1 |
| Health behaviors                          | Total        | Male       | Female     | χ² (p-value) |
|------------------------------------------|--------------|------------|------------|--------------|
|                                          | n  | %    | n  | %    | n  | %    |              |              |
| Alcohol use                              | No | 927  | 62.2       | 370 | 63.2       | 557 | 61.5       | 0.47 (0.492) |
|                                          | Yes| 564  | 37.8       | 215 | 36.8       | 349 | 38.5       |
| Methamphetamine use                      | No | 1441 | 96.6       | 567 | 96.9       | 874 | 96.5       | 0.23 (0.634) |
|                                          | Yes| 50   | 3.4        | 18  | 3.1        | 32  | 3.5        |
| Heroine use                              | No | 1484 | 99.5       | 581 | 99.3       | 903 | 99.7       | 0.95 (0.331) |
|                                          | Yes| 7    | 0.5        | 4   | 0.7        | 3   | 0.3        |
| Opium use                                | Yes| 1440 | 96.6       | 559 | 95.6       | 881 | 97.2       | 3.01 (0.080) |
|                                          | No | 51   | 3.4        | 26  | 4.4        | 25  | 2.8        |
| Marijuana use                            | Yes| 1460 | 97.9       | 571 | 97.6       | 889 | 98.1       | 0.47 (0.495) |
|                                          | No | 31   | 2.1        | 14  | 2.4        | 17  | 1.9        |
| Tattooed                                 | No | 1317 | 88.3       | 464 | 79.3       | 853 | 94.2       | 75.88 (< 0.001*) |
|                                          | Yes| 174  | 11.7       | 121 | 20.7       | 53  | 5.8        |
| Ear piercing                             | No | 699  | 46.9       | 504 | 86.2       | 195 | 21.5       | 596.26 (< 0.001*) |
|                                          | Yes| 792  | 53.1       | 81  | 13.8       | 711 | 78.5       |
| History of blood transfusion             | No | 1446 | 97.0       | 565 | 96.5       | 881 | 97.2       | 0.53 (0.467) |
|                                          | Yes| 45   | 3.0        | 20  | 3.4        | 25  | 2.8        |
| History of organ transplantation         | No | 1468 | 98.5       | 573 | 97.9       | 895 | 98.8       | 1.64 (0.200) |
|                                          | Yes| 23   | 1.5        | 12  | 2.1        | 11  | 1.2        |
| History of medical surgery               | No | 1325 | 88.9       | 534 | 91.3       | 791 | 87.3       | 5.68 (0.017*) |
|                                          | Yes| 166  | 11.1       | 51  | 8.7        | 115 | 12.7       |
| Drug injection from unqualified health professional | No | 1442 | 96.7       | 559 | 95.6       | 883 | 97.5       | 4.06 (0.044*) |
|                                          | Yes| 49   | 3.3        | 26  | 4.4        | 23  | 2.5        |
| Acupuncture                              | No | 1377 | 92.4       | 544 | 93.0       | 833 | 91.9       | 0.55 (0.457) |
|                                          | Yes| 114  | 7.6        | 41  | 7.0        | 73  | 8.1        |
| Sharing toothbrush                       | No | 1384 | 92.8       | 527 | 90.1       | 857 | 94.6       | 10.84 (0.001*) |
|                                          | Yes| 107  | 7.2        | 58  | 9.9        | 49  | 5.4        |
| History of receiving HBV vaccine         | Yes| 78   | 5.2        | 113 | 19.3       | 140 | 15.5       | 4.55 (0.208) |
|                                          | No | 1059 | 71.0       | 313 | 53.5       | 515 | 56.8       |
|                                          | Not sure| 192 | 12.9       | 105 | 17.9       | 175 | 19.3       |
|                                          | Don't know| 162 | 10.9       | 54  | 9.2        | 76  | 8.4        |

*Significance level at α = 0.05
Results
A total of 1491 individuals were recruited into the analysis; 60.8% were females, 81.3% were aged between 30 and 60 years, and 86.0% were married. The majority were illiterate (54.9%), were Buddhist (55.7%), worked in agricultural sections (87.3%), and had an annual income of less than 50,000 baht per year (72.9%). A large proportion held Thai ID cards (95.2%) and had the right to access to free-of-charge care (95.0%) (Table 1).

One-third of the participants used alcohol (37.8%), but a few people used other substances: amphetamine (3.4%), heroin (0.5%), opium (3.4%), and marijuana (2.1%). More than half of the participants had experienced ear piercing (53.1%), and 11.3% were tattooed. A small proportion of individuals had experienced blood transfusion (3.0%), organ transplantation (1.5%), medical surgery (11.1%), and acupuncture (7.6%). Five (5) variables were found the statistical significances on the proportions in different health behaviors between sex; tattooed (p-value < 0.001), ear piercing (p-value < 0.001), history of medical surgery (p-value = 0.017), drug injection from unqualified health professional (p-value = 0.044), and sharing toothbrush p-value = 0.001 (Table 2).

Among the 1491 participants, 113 (7.6%) were positive for HBsAg, 19.2% were positive for anti-HBs, and 18.9% were positive for anti-HBc. The overall prevalence of hepatitis B infection was 26.6% (396 out of 1489). Moreover, three participants were positive for both anti-HBsAg and HBsAg. There were no statistical differences among the tribes on HBsAg, anti-HBs, and total anti-HBc status (Table 3).

Seven (7) variables were found the statistical significance in having hepatitis B infection; age (p-value = 0.021), tribe (p-value = 0.042), education (p-value = 0.006), occupation (p-value = 0.031), income (p-value = 0.008), history of having hepatitis B infection in family member (p-value = 0.049), and alcohol use (p-value = 0.042) (Table 4).

In the univariate analysis, five variables were found to be associated with hepatitis B infection: tribe, religion, occupation, income, and alcohol use. However, in the multivariate analysis, after controlling for sex, age, marital status, education, and ID card, only three (3) variables remained associated with hepatitis B infection: tribe, religion, and alcohol use. Those who were in the Yao and Lisu tribes had a 1.64-fold (95% CI = 1.08–2.49) and a 1.93-fold (95% CI = 1.10–3.41) greater chance, respectively, of HBV infection than did those in the Karen tribe. Those who were Christian had a 1.41-fold (95% CI = 1.06–1.87) greater chance of HBV infection than did those who were Buddhist. Those who did not use alcohol had a 1.29-fold (95% CI = 1.01–1.65) greater chance of HBV infection than did those who used alcohol (Table 4).

Discussion
The hill tribe people aged 25 years and over who do not fall in the targeted area of HBV vaccination under the Thailand national EPI program have a high prevalence of HBV infection (26.6%); this population also has a high prevalence of HBsAg carriers (7.6%). The prevalence has increased according to the increase of age and income. Several risk behaviors affect the hill tribe population, including the use of various substances that could lead to HBV infection. However, individuals in certain tribes (Yao and Lisu), Christians and those who did not use alcohol were at a greater risk of HBV infection than others were.

The prevalence of HBV infection among the hill tribe adult populations was 26.6%, and the hepatitis B

Table 3 Hepatitis B biomarkers in different tribes

| Biomarkers | Total | Akha | Lahu | Hmong | Yao | Lisu | Karen | χ² (p-value) |
|------------|-------|------|------|-------|-----|------|-------|--------------|
| HBsAg      |       |      |      |       |     |      |       |              |
| Positive   | 113   | 7.6  | 15.1 | 26    | 7.5 | 22   | 7.4  | 20           | 1.2          | 9   | 13.2 | 21   | 7.6 | 10.7 (0.056) |
| Negative   | 1378  | 92.4 | 84.9 | 120   | 92.5 | 97.7 | 92.5 | 174          | 88.0         | 59  | 86.8 | 295  | 81.3 |            |
| Anti-HBs   |       |      |      |       |     |      |       |              |
| Positive   | 286   | 19.2 | 66.6 | 22.4  | 59  | 17.1 | 49   | 16.4         | 37           | 22.2| 23.5 | 59   | 20.6 | 6.29 (0.278) |
| Negative   | 1205  | 80.8 | 33.4 | 77.6  | 287 | 82.9 | 287  | 83.6         | 130          | 77.8| 76.5 | 257  | 81.3 |            |
| Total anti-HBc |   |      |      |       |     |      |       |              |
| Positive   | 283   | 18.9 | 66.6 | 22.4  | 58  | 16.7 | 48   | 16.0         | 37           | 22.2| 23.5 | 58   | 18.4 | 7.07 (0.215) |
| Negative   | 1208  | 81.1 | 33.4 | 77.6  | 288 | 83.3 | 251  | 84.0         | 130          | 77.8| 76.5 | 258  | 81.6 |            |
| HBsAg-positive and anti-HBs-positive | 3 | 0.2  | 0.0  | 1.33  | 1   | 33.3 | 1.33 | 0.0          | 0            | 0   | 0.0  | 1    | 33.3 | N/A           |
| HBV infection | 396  | 26.6 | 81.0 | 20.5  | 84  | 21.2 | 70   | 17.7         | 57           | 14.4| 25   | 6.3  | 79   | 19.9 (N/A)   |
Table 4  Univariate and multivariate analyses in identification factors associated with hepatitis B infection

| Factors                      | Hepatitis B infection | $\chi^2$ (p-value) | OR 95% CI | p value | OR Adj 95% CI | p value |
|------------------------------|-----------------------|--------------------|-----------|---------|---------------|---------|
| Sex                          |                       |                    |           |         |               |         |
| Male                         | Yes 150 (25.6)        | 435 (74.4)         | 0.42 (0.519) | 0.93   | 0.73–1.17     | 0.519   |
| Male                         | No 246 (27.2)         | 660 (72.8)         | 1.00      |         |               |         |
| Age (years)                  |                       |                    |           |         |               |         |
| < 30                         | Yes 18 (27.3)         | 48 (72.3)          | 7.72 (0.021<sup>a</sup>) | 1.00   |               |         |
| 30–60                        | Yes 338 (27.9)        | 874 (72.1)         | 1.03      | 0.59–1.80 | 0.914         | 1.00    |
| > 60                         | Yes 40 (30.1)         | 173 (69.9)         | 0.62      | 0.33–1.17 | 0.140         | 1.00    |
| Tribe                        |                       |                    |           |         |               |         |
| Akha                         | Yes 81 (27.5)         | 214 (72.5)         | 11.50 (0.042<sup>a</sup>) | 1.14   | 0.79–1.63     | 0.490   |
| Akha                         | No 84 (24.3)          | 262 (75.7)         | 0.96      | 0.68–1.37 | 0.829         | 0.91    |
| Lahu                         | Yes 170 (27.9)        | 482 (72.1)         | 1.03      | 0.63–1.33 | 0.664         | 0.99    |
| Lahu                         | No 57 (34.1)          | 110 (65.9)         | 1.56      | 1.03–2.34 | 0.034<sup>a</sup> | 1.64    |
| Yao                          | Yes 25 (36.8)         | 43 (63.2)          | 1.74      | 1.00–3.04 | 0.049<sup>b</sup> | 1.93    |
| Yao                          | No 20 (25.0)          | 237 (75.0)         | 1.00      |         |               |         |
| Religious status             |                       |                    |           |         |               |         |
| Married                      | Yes 18 (24.7)         | 55 (75.3)          | 5.42 (0.066) | 1.00   |               |         |
| Married                      | No 353 (27.5)         | 929 (72.5)         | 1.16      | 0.67–2.01 | 0.592         | 1.00    |
| Ever married                 | Yes 25 (18.4)         | 111 (81.6)         | 0.69      | 0.35–1.37 | 0.286         |         |
| Education                    |                       |                    |           |         |               |         |
| Illiterate                   | Yes 198 (24.2)        | 621 (75.8)         | 12.28 (0.006<sup>a</sup>) | 0.83   | 0.48–1.42     | 0.496   |
| Illiterate                   | No 100 (26.1)         | 283 (73.9)         | 0.92      | 0.52–1.62 | 0.768         |         |
| Secondary school             | Yes 78 (35.9)         | 139 (64.1)         | 1.46      | 0.81–2.62 | 0.206         |         |
| Secondary school             | No 20 (27.8)          | 52 (72.2)          | 1.00      |         |               |         |
| Having Thai ID card           |                       |                    |           |         |               |         |
| Having Thai ID card           | Yes 373 (26.3)        | 1047 (73.7)        | 1.30 (0.254) | 1.00   |               |         |
| Having Thai ID card           | No 23 (32.4)          | 48 (67.6)          | 1.35      | 0.81–2.24 | 0.156         |         |
| Having free access to health care services |                       |                    |           |         |               |         |
| Having free access to health care services | Yes 376 (26.5) | 1044 (73.5) | 0.10 (0.753) | 1.00   |               |         |
| Having free access to health care services | No 20 (28.2) | 51 (71.8) | 0.92 | 0.54–1.56 | 0.761         |         |
| Occupation                   |                       |                    |           |         |               |         |
| Farmer                       | Yes 345 (24.5)        | 957 (75.5)         | 6.97 (0.031<sup>a</sup>) | 1.00   |               |         |
| Farmer                       | No 16 (44.4)          | 20 (55.6)          | 2.22      | 1.14–4.33 | 0.019<sup>a</sup> |         |
| Daily wage employee          | Yes 35 (22.9)         | 118 (77.1)         | 0.82      | 0.55–1.22 | 0.335         |         |
| Number of family members     |                       |                    |           |         |               |         |
| Number of family members     | < 4 202 (27.9)        | 523 (72.1)         | 1.39 (0.499) | 1.00   |               |         |
| Number of family members     | 4–7 153 (25.7)        | 443 (74.3)         | 0.89      | 0.70–1.14 | 0.372         |         |
| Number of family members     | > 7 41 (24.1)         | 129 (75.9)         | 0.82      | 0.56–1.21 | 0.324         |         |
| Number of family members     |                       |                    |           |         |               |         |
| Income                       | < 10,000              | 45 (28.5)          | 113 (71.5) | 9.65 (0.008<sup>a</sup>) | 1.00   |         |
| Income                       | 10,001 – 50,000       | 222 (23.9)         | 707 (76.1) | 0.78   | 0.54–1.15     | 0.217   |
| Income                       | > 50,000              | 129 (31.9)         | 275 (68.1) | 1.18   | 0.79–1.76     | 0.427   |

<sup>a</sup> Significant at the 0.05 level.
Table 4: Univariate and multivariate analyses in identification factors associated with hepatitis B infection (Continued)

| Factors                                      | Hepatitis B infection | $\chi^2$ | OR  | 95% CI | $p$-value | OR | 95% CI | $p$-value |
|----------------------------------------------|-----------------------|----------|-----|--------|-----------|----|--------|-----------|
|                                              | Yes n (%)             | No n (%) |     |        |           |    |        |           |
| History of having hepatitis B vaccination    |                       |          |     |        |           |    |        |           |
| Yes                                          | 65 (25.7)             | 188 (74.3) | 3.52 | 0.318  | 1.00      |    |        |           |
| No                                           | 227 (27.4)            | 601 (72.6) | 1.09 | 0.79–1.51 | 0.589    |    |        |           |
| Not sure                                     | 78 (27.9)             | 202 (72.1) | 1.12 | 0.76–1.64 | 0.573    |    |        |           |
| Don’t know                                   | 26 (20.0)             | 104 (80.0) | 0.72 | 0.43–1.21 | 0.216    |    |        |           |
| History of having hepatitis B infection in family member |               |          |     |        |           |    |        |           |
| Yes                                          | 24 (30.8)             | 54 (29.2) | 7.84 | 0.049* | 1.00      |    |        |           |
| No                                           | 278 (26.3)            | 781 (72.7) | 0.80 | 0.49–1.32 | 0.384    |    |        |           |
| Not sure                                     | 62 (32.3)             | 130 (67.7) | 1.07 | 0.61–1.89 | 0.808    |    |        |           |
| Don’t know                                   | 32 (19.8)             | 130 (80.2) | 0.55 | 0.29–1.03 | 0.061    |    |        |           |
| Tattooed                                     |                       |          |     |        |           |    |        |           |
| Yes                                          | 345 (26.2)            | 972 (73.8) | 0.76 | 0.382  | 1.00      |    |        |           |
| No                                           | 51 (29.3)             | 123 (70.7) | 1.17 | 0.82–1.66 | 0.382    |    |        |           |
| Ear pierced                                  |                       |          |     |        |           |    |        |           |
| Yes                                          | 183 (26.2)            | 516 (73.8) | 0.10 | 0.756  | 1.00      |    |        |           |
| No                                           | 213 (26.9)            | 579 (73.1) | 1.04 | 0.82–1.31 | 0.756    |    |        |           |
| History of blood transfusion                 |                       |          |     |        |           |    |        |           |
| Yes                                          | 384 (26.6)            | 1062 (73.4) | 0.00 | 0.987  | 1.00      |    |        |           |
| No                                           | 12 (26.7)             | 33 (73.3)  | 1.01 | 0.51–1.97 | 0.987    |    |        |           |
| History of organ transplantation             |                       |          |     |        |           |    |        |           |
| Yes                                          | 391 (26.6)            | 1077 (73.4) | 0.28 | 0.598  | 1.00      |    |        |           |
| No                                           | 5 (38.5)              | 18 (61.5)  | 0.77 | 0.28–2.08 | 0.599    |    |        |           |
| History of medical surgery                   |                       |          |     |        |           |    |        |           |
| Yes                                          | 350 (26.4)            | 975 (73.6) | 0.13 | 0.722  | 1.00      |    |        |           |
| No                                           | 46 (27.7)             | 120 (72.3) | 1.07 | 0.74–1.53 | 0.722    |    |        |           |
| Receiving a drug injection from an untrained medical professional |       |          |     |        |           |    |        |           |
| Yes                                          | 387 (26.8)            | 1055 (73.2) | 1.74 | 0.187  | 1.00      |    |        |           |
| No                                           | 9 (27.4)              | 40 (72.6)  | 0.61 | 0.30–1.28 | 0.191    |    |        |           |
| Having acupuncture                           |                       |          |     |        |           |    |        |           |
| Yes                                          | 363 (26.4)            | 1014 (73.6) | 0.36 | 0.548  | 1.00      |    |        |           |
| No                                           | 33 (28.9)             | 81 (71.1)  | 1.14 | 0.75–1.74 | 0.548    |    |        |           |
| Sharing toothbrush                           |                       |          |     |        |           |    |        |           |
| Yes                                          | 365 (34.6)            | 1019 (65.4) | 0.34 | 0.558  | 1.00      |    |        |           |
| No                                           | 31 (28.9)             | 76 (71.1)  | 1.14 | 0.74–1.76 | 0.558    |    |        |           |
| Alcohol use                                  |                       |          |     |        |           |    |        |           |
| Yes                                          | 133 (23.6)            | 431 (76.4) | 4.12 | 0.042* | 1.00      |    |        |           |
| No                                           | 263 (28.4)            | 664 (71.6) | 1.28 | 1.01–1.63 | 0.043* | 1.29 | 1.01–1.65 | 0.040* |
| Methamphetamine use                          |                       |          |     |        |           |    |        |           |
| Yes                                          | 11 (22.0)             | 39 (78.0)  | 0.55 | 0.458  | 0.77      | 0.77 | 0.39–1.53 | 0.459    |
| No                                           | 385 (26.7)            | 1056 (73.3) | 1.00 |        |           |    |        |           |
| Heroin use                                   |                       |          |     |        |           |    |        |           |
| Yes                                          | 3 (42.9)              | 4 (57.1)   | 0.96 | 0.328  | 2.08      | 0.46 | 9.34     | 0.338    |
| No                                           | 393 (26.5)            | 1091 (73.5) | 1.00 |        |           |    |        |           |

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Another study conducted in India [37] reported that HBV infection was higher than the national rate that individuals in some tribes in Malaysia had a rate of were. Moreover, a study in Malaysia [36] also reported that some tribes living in rural areas were at a significantly greater risk of HBV than others. This finding coincides with the findings of a study in Iran [35], which reported that some tribes living in rural areas were at a significantly greater risk of HBV than others were. Moreover, a study in Malaysia [36] also reported that individuals in some tribes in Malaysia had a rate of HBV infection that was higher than the national rate. Another study conducted in India [37] reported that different tribes who were living in eastern India had significantly different rates of HBV infection. A study in the United States [38] also demonstrated that some groups of people had a greater risk of HBV infection than others did. Apidechkul [20] also confirmed that some hill tribe youths had a greater chance of HBV infection than others did. While having looked more closely into some behaviors such as alcohol use, tattooed, and ear piecing, it was found that the Lisu and Yao.

In the hill tribe population, adults who were Christians had a greater risk of hepatitis B infection than did those who were Buddhists. A study in Pakistan [39] reported that in some cultures, a reliance on religion may serve as a barrier to obtaining HBV vaccines, resulting in HBV infection. A study among Nigerian pregnant women [40] found that those following different religions had significantly different rates of HBV infection. In Thailand there is no evidence presented on restriction to access EPI program based on their religion. However, after taking a closer look into some variables in the participants’ background, it was found that those who were Christians had a lower education that those who were Buddhists significantly ($p$-value < 0.001). This might be making them access to EPI program in different proportions, and resulting to have different risks of hepatitis B infection. This association was supported by a study in China [41] and Malaysia [42].

Among the hill tribe adults, those who did not use alcohol had a greater risk of HBV infection than did those who used alcohol. This finding is not consistent with that of a study conducted in Korea [43], which clearly presented that those who had low levels of education tended to consume alcohol and develop HBV infection later. There are few studies on the association between alcohol use and HBV infection, but there are clear explanations for the association between alcohol use among

Table 4 Univarite and multivariate analyses in identification factors associated with hepatitis B infection (Continued)

| Factors                  | Hepatitis B infection | $\chi^2$ (p-value) | OR 95% CI  | $p$-value | OR Adj 95% CI  | $p$-value |
|--------------------------|-----------------------|---------------------|------------|----------|----------------|----------|
| Yes                      | 13 (54.5)             | 38 (74.5)           | 0.03 (0.860) | 0.94     | 0.50–1.79      | 0.860    |
| No                       | 383 (26.6)            | 1057 (73.4)         | 1.00       |          |                |          |

Marijuana use

| Yes                      | 5 (16.1)              | 26 (83.9)           | 1.77 (0.184) | 0.53     | 0.20–1.38      | 0.191    |
| No                       | 391 (26.8)            | 1069 (73.2)         | 1.00        |          |                |          |

*Significance level at α = 0.05
those with hepatitis and the development of cirrhosis and hepatocellular carcinoma. Alcohol use might not be the main route of HBV transmission among the hill tribe people in Thailand.

In our study, no participants refused to take part in the study. All participants who were positive for HBV biomarkers were informed to visit a medical doctor for proper treatment and care. Moreover, those who had a negative result for all HBV biomarkers were also asked to obtain the HBV vaccine from a health care provider properly and in a timely manner. The pattern of assessing exposures and outcomes in the same time on a cross-sectional study, it could impact the associations between variables while making interpretations [44]. Therefore, to make more clear on these associations, further stronger study designs to work out is recommended.

**Conclusion**
The hill tribe adult population aged 25 years and over with HBV who have never been immunized under the Thai national EPI program have low levels of education, live in poor economic conditions and reside in areas of Thailand with a high prevalence of hepatitis B virus. They are highly vulnerable to hepatitis B infection, particularly those who are not using alcohol, Christians and those belonging to certain tribes, namely, the Lisu and Yao tribes. Effective public health interventions, including providing this population with HBV vaccines, need to be considered and implemented to reduce the HBV infection rate, save lives and decrease other medical costs.

**Abbreviations**
anti-HBc: Hepatitis B core antibody; anti-HBs: Hepatitis B surface antibody; CDC: Centers for Disease Control and Prevention; CI: Confident interval; EPI: Expanded Program on Immunization; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; ID: Identification card; IOC: Item-Objective Congruence; SD: standard deviation; WHO: World Health Organization

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**Authors’ contributions**
PU: designed the study, collected data, laboratory analysis, analyzed data, and final proved manuscript; TA: designed the study, collected data, analyzed data, drafted manuscript, and final proved manuscript; RT, CC, FY: contacted the study settings, collected data, and final proved manuscript.

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**Availability of data and materials**
Data file is attached as supplement file. Additional data supporting these findings are available by personal request at Tawatchai_api@mfu.ac.th.

**Ethics approval and consent to participate**
All research protocols and tools were approved by the Chiang Rai Provincial Public Health Research Ethical Committee (No. CRPHO 17/2562). All participants were asked to obtain written informed consent form after oral explanation on all essential information of the study, before starting of data collection.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

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