

High Prevalence of Hepatitis B Virus Infection in Young Adults in Ternate, Eastern Indonesia

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Abstract. The incidence of hepatitis B virus (HBV) infection has been declining thanks to the universal hepatitis B infant immunization program. Nevertheless, young adults born before the program implementation might have acquired HBV in early childhood or remain susceptible to infection. This study aimed to evaluate hepatitis B epidemiology in asymptomatic young adult population in Ternate, eastern Indonesia. Serum samples of 376 subjects (aged 17–25, mean 19.82 ± 1.69 years; male/female 138/238) were screened for HBV parameters serologically (HBV surface antigen [HBsAg]; its antibody [anti-HBs]; anti-core antigen [anti-HBc]), and molecularly (HBV DNA). HBsAg, anti-HBc, anti-HBs, and HBV DNA prevalence were 15.7%, 36.2%, 24.2%, and 27.9%, respectively, with male predominance. Of all subjects, 13.0% were HBsAg negative with detectable HBV DNA (occult hepatitis B [OHB]), and 56.4% showed negativity for all seromarkers. This population showed high hepatitis B prevalence with substantial occurrence of OHB. However, a high percentage of the population were still susceptible and at risk of HBV infection. This study emphasizes the necessity to improve prevention strategies to screen and manage HBV carriers, including the adoption of catch-up or booster vaccination targeted to young adult populations. Investigations on the roles of host-virus interactions associated with OHB and its implications are warranted.

INTRODUCTION

Hepatitis B virus (HBV) infection is a major public health problem. Worldwide, approximately 2 billion people have been infected, and more than 240 millions are chronic carriers at risk of developing progressive liver diseases such as cirrhosis, liver failure, and hepatocellular carcinoma (HCC).^{1,2} HBV infection accounts for more than 780,000 deaths each year, with HCC currently being the fifth most frequent cancer and the second most common cause of cancer mortality.^{1,3} By 2010, HBV is responsible for around 45% cases of HCC and 30% of cirrhosis, with much higher proportions in low- and middle-income countries (LMICs).^{3,4} Based on the significant public health burden, it is essential to prevent HBV transmission by vaccination, early detection, and treatment of infected individuals to achieve and maintain disease resolution.²

The species-specific HBV spreads by direct contact with infectious blood or body fluids.^{1,2} HBV screening is commonly done by serological testing for viral markers and its host antibodies, including the hepatitis B surface antigen (HBsAg), its antibody (anti-HBs), and antibody against the HBV core antigen (anti-HBc).⁵ HBsAg is excessively formed during the production of new viral particles,⁶ making it a good HBV screening parameter.⁵ Persistence of HBsAg detection after a period of 6 months or more is defined as chronic hepatitis B.^{7–9} On the other hand, anti-HBs is considered as the protective antibody, because of its HBsAg-neutralizing property.⁶ Anti-HBs can be produced as a result of resolved HBV infection or induction by vaccination.^{1,10} Meanwhile, anti-HBc (IgM anti-HBc followed by IgG anti-HBc) is the first antibody produced in immune-competent individuals during natural infection, rendering it as a main indicator of HBV exposure, either as ongoing or resolved infection.⁶

With the advent of sensitive molecular detection techniques, better HBV screening can be performed. However, its results are often in disagreement with the serological results.^{11–13} Such discrepancies are of public health significance, since HBV DNA is detected alongside HBsAg-negative serological profiles that are previously considered to be noninfectious, including isolated anti-HBc and seronegative samples.^{14–17} Commonly, low HBV DNA titer is detectable in these samples, classifying them as occult hepatitis B (OHB).^{12,18}

Indonesia is categorized as a moderately to highly endemic region for hepatitis B.^{1,19} The HBsAg detection rate is not uniform across the Indonesian archipelago, with higher prevalence in the eastern than the western region.^{20,21} It is worthy to note that Indonesia presents an exceptionally diverse host ethnicity backgrounds, a condition that supports the emergence of new HBV subgenotypes as well as unique HBV variants.^{21,22} Ternate as one of the many uniquely located historical crossroads of human migration and trade between mainland Asia and Australia (Figure 1),^{23,24} is a good example to study the effects of host heterogeneity in supporting the development of unusual serological and/or molecular HBV profiles.

A nationwide vaccination program has been implemented in Indonesia since 1997.²⁵ It is of interest to see the condition against which this vaccination program is challenging, with an epidemiology study on the population of young adults who were born before the implementation of the program. These individuals can be considered as the “missing generation” in term of hepatitis B management. Young adults—especially adolescents—are in the age group with the tendency of risky behavior, putting them at higher risk of HBV exposure.²⁶ In addition, from the socioeconomic point of view, the study population of Ternate can be considered as a representative for other communities in eastern Indonesia, which commonly have less access to health care, because of their relative isolations in small islands or remote areas, in combination with lower gross domestic product per capita.^{27–29}

This study was carried out to determine the epidemiology of HBV infection in young adult population of Ternate, both

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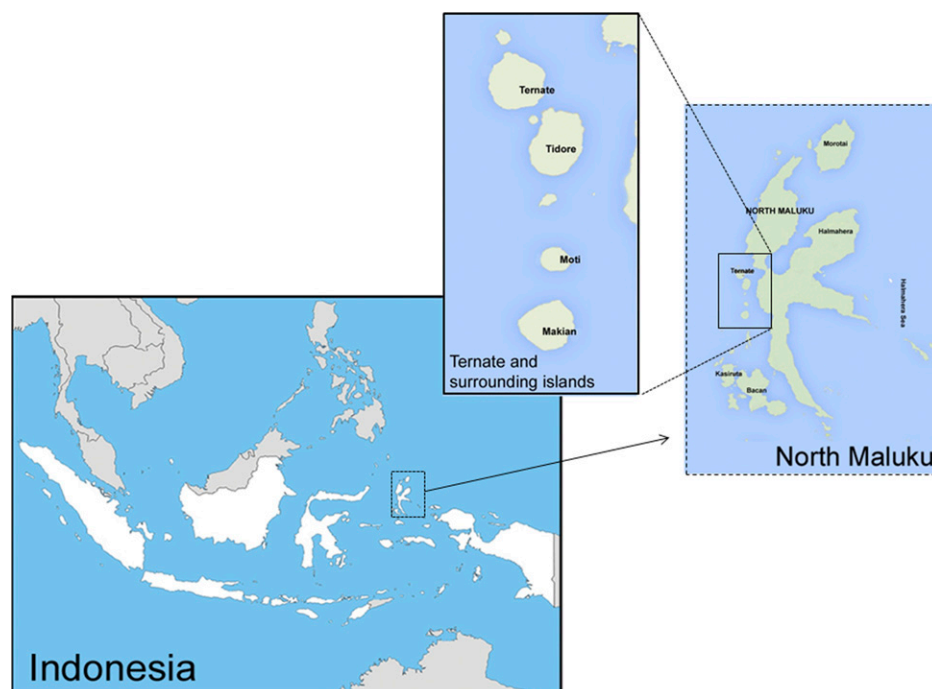


FIGURE 1. Map of Ternate, Indonesia. The position and relative isolation of Ternate and its surrounding islands amidst the North Maluku islands (or North Moluccas) of eastern Indonesia, which are located in the transitional biogeographical zone between the Sunda (Asia) and Sahul (Australia) lands.

by serological parameters and HBV DNA detection. Results of this study may exemplify the magnitude of HBV problem in young adults as an important segment of the population who will enter the adult community, particularly in the eastern part of Indonesia. This study will also provide practical information that may contribute to the improvement of efficient prevention and early detection to curb the ramification of hepatitis B in the society.

MATERIALS AND METHODS

Study population. A total of 376 participants (aged 17–25, mean 19.8 ± 1.7 years; male/female 138/238) who were students in high schools and colleges in Ternate were recruited in 2010. They constituted of several ethnic populations from Ternate and its surrounding islands (Figure 1), in the North Maluku islands. All participants were considered as healthy asymptomatic subjects with no recorded clinical signs and symptoms related to liver diseases. Vaccination history of participants was unavailable. Serum samples were collected and transported to the Eijkman Institute in Jakarta and stored at -30°C until use. Individual written informed consent for study involvement was obtained from all participants. The study protocol was in accordance with and approved by the Eijkman Institute Research Ethics Commission (EIREC 32/2008).

Serological assays. Serum samples were subjected to HBsAg, anti-HBs, and anti-HBc immunoassays (Evolis™ Biorad Laboratories, Inc., Hercules, CA) according to the manufacturer's instruction. Anti-HBs quantification was performed using Ausab Anti-HBs Quantitation Panel (Abbott Laboratories, Chicago, IL), with concentrations ≥ 10 IU/L considered positive.

HBV DNA extraction and amplification. To isolate HBV DNA, 100 μL of each serum sample was subjected to Trizol-LS

extraction (Invitrogen, Carlsbad, CA). The resulting precipitate was resuspended in 30 μL of double-distilled water and stored at -20°C . Nested polymerase chain reaction (PCR) using two sets of primers (S2-1/S1-2 [5'-CAA GGT ATG TTG CCC GTT TG-3'/5'-CGA ACC ACT GAA CAA ATG GC-3'] and S088/S2-2 [5'-TGT TGC CCG TTT GTC CTC TA-3'/5'-GGC ACT AGT AAA CTG AGC CA-3']) was performed targeting an S gene segment encoding the “a” determinant of HBsAg.^{30–34} Denaturation, annealing, and extension were done at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 90 seconds/1 minute for the first/second PCR rounds (35/30 cycles), respectively. This nested PCR method was capable of detecting HBV DNA at very low titer (< 6 IU/mL), as validated using a panel of sera with known HBV DNA titers based on quantitative real-time PCR (Cobas TaqMan™ HBV Test; Roche Diagnostics, Indianapolis, IN) (Supplemental Table 1). The amplification products were visualized on ethidium bromide-stained 2% agarose gel under ultraviolet light. Kwok and Higuchi rules were followed strictly in all experimental steps to avoid cross-contamination.³⁵

Statistical analysis. The baseline data were descriptively summarized. Statistical analyses were performed using Statistical Package for Social Sciences v.20 (SPSS Inc., Chicago, IL), with levels of association assessed by χ^2 test. Correlations between HBV DNA detection and serological screening parameters were evaluated by Spearman rank correlation test. All statistical significance values were assessed at $P < 0.05$.

RESULTS

Serological profiles of HBV infection. Of 376 subjects, HBsAg, anti-HBc, and anti-HBs were positive in 59 (15.7%), 136 (36.2%), and 91 (24.2%), respectively (Table 1). Regardless

TABLE 1
Hepatitis B serology and HBV DNA detection of Ternate isolates

HBV serology						HBV DNA detection	
Parameters							
HBsAg	Anti-HBc	Anti-HBs	<i>N</i> *	%*	Interpretation	<i>n</i> †	%†
+	+	+	5	1.3	Chronic hepatitis B	4	80.0
+	+	−	31	8.2	Chronic hepatitis B	31	100.0
+	−	+	1	0.3	Possible HBsAg mutant	0	0.0
+	−	−	22	5.9	Isolated HBsAg	21	95.4
−	+	+	80	21.3	Resolved from infection	38	47.5
−	+	−	20	5.3	Possible occult infection	5	25.0
−	−	+	5	1.3	Immune due to vaccination	0	0.0
−	−	−	212	56.4	Susceptible	6	2.8
TOTAL			376	100.0		105	27.9

HBV = hepatitis B virus; HBV DNA = hepatitis B virus DNA; HBsAg = hepatitis B surface antigen; anti-HBc = antibody against hepatitis B core antigen; anti-HBs = antibody against hepatitis B surface antigen; PCR = polymerase chain reaction.

*The number of samples among the total study population according to the serological profile, together with its percentage.

†The number of samples positive for HBV DNA detection using nested PCR method, together with its percentage within the corresponding serological profile group (n/N).

of anti-HBs status, of the 59 HBsAg-positive subjects, 36 (61.0%) were anti-HBc positive and 23 (39.0%) were anti-HBc negative, of whom 22 had isolated HBsAg profile. Of 136 anti-HBc-positive subjects, 36 (26.5%) were HBsAg positive, and 100 (73.5%) were HBsAg negative, including 20 isolated anti-HBc cases who had no anti-HBs. Of 91 anti-HBs-positive subjects, 1 (1.1%) had HBsAg, 80 (87.9%) had anti-HBc, 5 (5.5%) had concurrent HBsAg and anti-HBc, and 5 (5.5%) had exclusive anti-HBs positivity. A further breakdown of the anti-HBs-positive subjects showed that 34 (37.4%) had anti-HBs at titers 10–100 IU/L (low titer), 25 (27.5%) at 100–500 IU/L (medium titer), 8 (8.8%) at 500–1,000 IU/L (high titer), and 24 (26.4%) at $\geq 1,000$ IU/L (very high titer). A total of 212 (56.4%) individuals were seronegative for all hepatitis B serological markers.

Molecular detection of HBV DNA. Overall, HBV DNA was found in 105 (27.9%) samples (Table 1). A total of 35 (97.2%) HBsAg-/anti-HBc-positive isolates had detectable HBV DNA, with the one negative sample had concurrent HBsAg-/anti-HBc-/anti-HBs-positive profile. Further, 43 (40.9%) of HBV DNA-positive samples were HBsAg-negative but anti-HBc positive, indicating occult HBV infection.¹⁸ Out of the 22 isolated HBsAg samples, 21 (95.4%) were also HBV DNA positive, with the one negative sample showing very low HBsAg reactivity—marginally outside the gray zone area (data not shown). The HBsAg-/anti-HBs-positive sample was found to be HBV DNA negative. Likewise, no HBV DNA was found in samples with isolated anti-HBs profile. Interestingly, 6 (2.8%) seronegative samples were found to be HBV DNA positive. All of the OHB samples were positive for HBV DNA only in the second round of nested PCR detection, indicating very low HBV DNA titers ($< 2.6 \times 10^3$ IU/mL). We also observed that the proportions of HBV DNA detection and OHB were higher in subjects with positive anti-HBs, as depicted in Figure 2.

Correlations between gender, age, serological profiles, and HBV DNA detection. HBsAg, anti-HBc, and HBV DNA prevalence were found to be higher in males than females (25.4% versus 10.1%, $P < 0.001$; 47.8% versus 29.4%, $P = 0.001$; 41.3% versus 20.2%, $P < 0.001$, respectively). No significant difference was found in anti-HBs prevalence (male 29.0%, female 21.4%, $P = 0.106$). Looking at the complete serological profile, more females were categorized as seronegative individuals (63.9% versus 43.5%, $P < 0.001$). On the other hand, HBsAg-/anti-HBc-positive profile was more

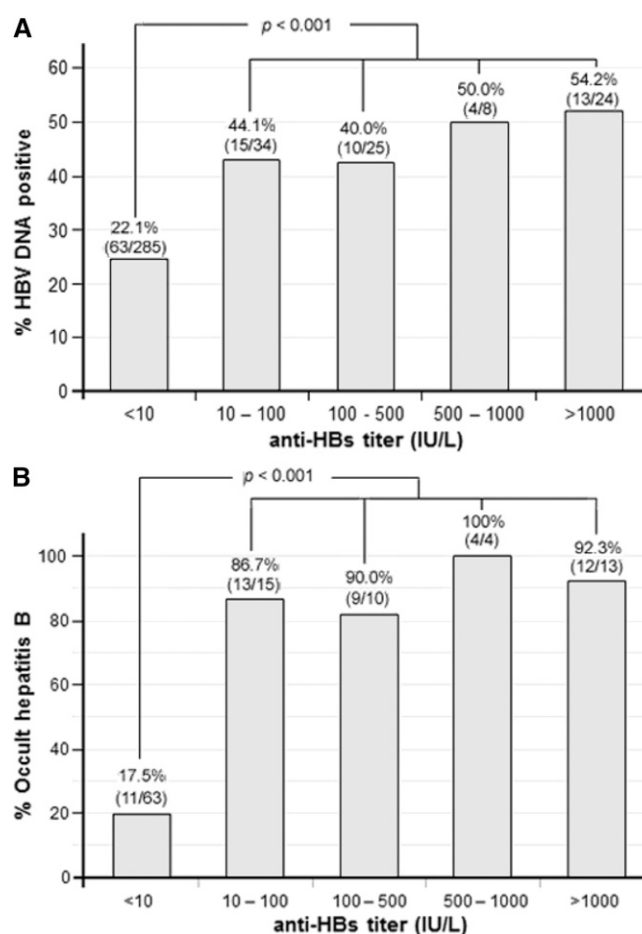


FIGURE 2. Distribution of (A) hepatitis B virus DNA (HBV DNA) positivity and (B) occult hepatitis B cases in relation to anti-HBs titers. The 376 samples in this study were classified into five groups: negative anti-HBs (anti-HBs < 10 IU/L), low (≥ 10 –100 IU/L), medium (≥ 100 –500 IU/L), high (≥ 500 –1,000 IU/L), and very high ($\geq 1,000$ IU/L) anti-HBs titers. It was observed that HBV DNA was detected with significantly higher percentage (46.1% [42/91]) in anti-HBs-positive than in anti-HBs-negative (22.1% [63/285]) subjects ($P < 0.01$), regardless of anti-HBs titers (A). As illustrated in B, most of these cases were attributable to occult hepatitis B (OHB), with significantly higher percentage in anti-HBs-positive in comparison to anti-HBs-negative subjects (90.5% [38/42] vs. 17.5% [11/63]; $P < 0.01$).

prevalent in males (13.8% versus 5.04%, $P = 0.006$). No significant difference was observed in other serological profiles.

Age wise, individuals with positive HBsAg, anti-HBc, anti-HBs, or HBV DNA were slightly older (mean age 20.7 years versus 19.6 years, $P < 0.001$; 20.4 years versus 19.5 years, $P < 0.001$; 20.1 years versus 19.7 years, $P = 0.046$; 20.4 years versus 19.6 years, $P < 0.001$, respectively). Seronegative profile was observed in somewhat younger individuals (19.4 years versus 20.4 years, $P < 0.001$), whereas positive HBsAg/anti-HBs/anti-HBc, HBsAg/anti-HBc, isolated HBsAg, and isolated anti-HBc profiles were associated with older mean age (21.8 years versus 19.8 years, $P = 0.008$; 20.5 years versus 19.8 years, $P = 0.017$; 20.9 years versus 19.8 years, $P = 0.003$; 20.9 years versus 19.8 years, $P = 0.003$, respectively). No age correlation was observed in other serological profiles.

HBV DNA detection had better correlation with HBsAg than with anti-HBc, anti-HBs, or concurrent HBsAg/anti-HBc status ($r = 0.644$ versus 0.494, 0.230, or 0.482, respectively; all with $P < 0.001$). Seronegative profile was associated with HBV DNA negativity ($P < 0.001$). In contrast, positive HBsAg/anti-HBs/anti-HBc, HBsAg/anti-HBc, isolated HBsAg, and anti-HBs/anti-HBc profiles were associated with positive HBV DNA detection ($P = 0.009$, $P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively). No association was observed between HBV DNA detection and other serological profiles. No association was observed between HBV DNA positivity and gender or age in association with specific serological profile.

DISCUSSION

Hepatitis B as a worldwide health problem is not evenly distributed, with most endemic regions located in largely LMICs of Asia and Africa.^{1,2,36} So far, hepatitis B prevention focuses on infant immunization program, as has been implemented in 183 World Health Organization (WHO) Member States, including Indonesia.^{1,36} This program provides protection to virtually the entire population born each year, successfully reducing hepatitis B prevalence as reported in several countries.^{37–39} However, it would take about 15–20 years to obtain whole community protection using this strategy alone.^{40,41} Adolescents and young adults who were born before the initiation of universal infant immunization program would remain susceptible to HBV infection; many might even have acquired the disease in early childhood and are at risk of developing advanced chronic diseases later in their life.^{2,42} This population segment could be regarded as the “missing generation,” with no currently available clinical guidelines targeting hepatitis B immunization to this age group, while they belong to the cohort entering the adult communities and may engage in activities that put them at risk for HBV infection.^{2,26,41}

Implications of chronic liver diseases in this generation will result in significant socioeconomic burden, particularly when they enter productive ages, both by the loss of effective working individuals and the increase of health-related costs.^{26,42} In addition, they also play important roles in HBV transmission for their entire lifetime: both vertically from parents to children, and horizontally to the community.^{26,40–43} This study intended to analyze the disease burden in young adults of Ternate and its implications as an input in hepatitis B management, especially in communities that experience difficulties in

accessing health facilities—both because of their relative isolation and lower socioeconomic condition.

Based on the population baseline characteristics, it was found that most parameters related to HBV infectivity were associated with male and older age, as observed elsewhere.^{44,45} Serologically, this study population demonstrated high hepatitis B prevalence with 15.7% HBsAg detection rate. Moreover, the anti-HBc prevalence showed that more than one-third (36.2%) of the population had been exposed to HBV. In contrast, anti-HBs was found only in 24.2% of the studied population, 93.4% of which concurrently with anti-HBc, indicating exposure and resolution rather than immunization. Using molecular detection, HBV DNA was found almost twice more often than the HBsAg prevalence (27.9% versus 15.7%). These findings showed high infection rate among the studied population. This is of concern, because it occurs in young subjects that are usually in the immune-tolerant phase of chronic hepatitis B with normal physical and laboratory examination, but at risks of developing advanced liver diseases or liver cancer, with lifetime potentials as source of HBV infection.^{1,2,8,26} It is also worthy to note that 56.4% of the study population showed seronegativity for all hepatitis B serological markers, indicating susceptibility to HBV infection. It draws attention to the urgent necessity to reevaluate hepatitis B prevention and control strategy, particularly in protecting susceptible individuals against the environment with potentially high HBV transmission.

Generally, screening of HBV infection relies on the serological detection of HBsAg.⁵ However, in this study, using molecular detection of HBV DNA, discrepancies between the two methods were observed. There were subjects who were HBsAg positive without HBV DNA detection. Several factors might be involved: 1) the window period of seroconversion before complete clearance by circulating anti-HBs, 2) very low HBV replication rate, or 3) the unique HBsAg synthesis that may persistently form “empty” HBsAg particles without active viral replication.^{14,46,47} Further HBsAg quantification analysis and liver biopsy would be needed for such an inquiry. More importantly, this study found a high rate (13.0%) of OHB, characterized by HBsAg negativity but having detectable HBV DNA; these cases were attributable to anti-HBs/anti-HBc-positive samples (77.5%), isolated anti-HBc samples (10.2%), and seronegative samples (12.2%). The detection of HBV DNA in 2.8% of seronegative samples, which was higher than previously reported, should be of concern.^{15,48} It is also noteworthy that 52.5% of the anti-HBs/anti-HBc-positive samples were HBV DNA negative, underlining the high rate of resolved infection in this population.

Overall, HBV DNA detection was most strongly associated with HBsAg positivity, followed by anti-HBc alone, then concurrent HBsAg/anti-HBc. These results support the use of HBsAg as the common marker for initial screening of HBV infection, as performed in blood donation centers in Indonesia. However, the true prevalence of HBV could be underestimated due to the presence of OHB cases.^{12,13,18} When this situation is applied in blood banking, the high OHB prevalence might consequently cause the release of infectious blood units to recipients.

In countries such as Austria, Germany, and Japan, anti-HBc and anti-HBs are used as screening parameters for blood donation; blood units with positive anti-HBc and concurrent

anti-HBs at titers > 100 IU/L are considered safe.^{49,50} However, if this strategy is applied in the studied population, the use of anti-HBc would result in the disposal of 36.2% of blood units, with 42.6% of them might actually be safe. The anti-HBs titers in the HBsAg-negative/anti-HBc-positive samples did not illustrate the absence of viral infection, as 65.8% of anti-HBc/anti-HBs-positive OHB cases showed anti-HBs titers of > 100 IU/L (Figure 2). In general, these findings showed HBV DNA detection as a better screening method in determining HBV infectivity. However, its use for mass screening might present problems in cost-effectiveness and time-efficiency compared with serological assays. Therefore, in endemic countries such as Indonesia, it would still be more plausible to use HBsAg as the initial hepatitis B screening parameter, preferably complemented with anti-HBc, until such a time when molecular detection has become more affordable and applicable for general public use.

The epidemiological data derived in this study provide an initial assessment on the magnitude of the challenge that should be considered for the expansion of national immunization program. HBV protection profile in this population was found with relatively low prevalence of isolated anti-HBs (1.3%). However, these data should be viewed with discretion, due to the lack of immunization history. Furthermore, it might not pick up all immunologically protected individuals since anti-HBs titer wanes at different rates, and some of the seronegative subjects may actually have protective anti-HBs upon challenge by booster vaccination.^{51,52} On the positive side, none of these samples were HBV DNA positive, emphasizing the effectiveness of vaccination as community protection against hepatitis B. This also reiterates the need of catch-up and/or booster vaccination in adolescent and young adults, particularly those with seronegative hepatitis B markers, as an additional strategy besides the routine infant immunization as suggested in the newly published WHO guidelines.^{1,2,40,41,51}

The high occurrence of OHB in Ternate is another important outcome of this study. Several reports have suggested that occult HBV is transmissible through blood transfusion and organ transplantation, and may lead to cirrhosis and/or the subsequent development of HCC.⁵³ This finding is also of interest, because this area is positioned in the transitional zone between the Asian and Australian biogeographical regions, where parallel evolution and genotypic adaptation have occurred in organisms ranging from microbes to plants, invertebrates, vertebrates, and primates.²⁴ This might be considered in the explanation of the high OHB cases in this study, because differences in the HBV-specific host immune response have been described as one of the possible mechanisms leading to occult HBV,⁵⁴ as indicated by the presence of anti-HBs in 77.5% (38/49) of OHB cases in this study. Thus, this area offers a unique opportunity to investigate the implications of the host-virus interactions in determining the outcome of HBV infection.

In conclusion, we observed a high prevalence of hepatitis B in healthy young adult population of Ternate, both serologically by HBsAg (15.7%) and molecularly by HBV DNA detection (27.9%). The finding of high incidence of OHB (13.0%) is of concern, and should be taken into account in negotiating a strategy to screen and treat these individuals. Large proportion of the studied population still showed susceptibility to HBV infection, indicating the necessity to enhance

public awareness and the importance of catch-up or booster hepatitis B vaccination. The result of this study emphasizes the necessity to improve prevention strategies to screen and manage HBV carriers, including the adoption of catch-up or booster vaccination, particularly for high-risk target population like adolescents and young adults. Further studies are needed to illustrate a more complete picture of hepatitis B epidemiology in Indonesia and surrounding regions, with analysis of the roles of both host and viral factors to better understand the multiple aspects associated with OHB.

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