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GEOGRAPHICAL AND GENETIC DIVERSITY OF HEPATITIS B VIRUS IN THE WALLACEA, INDONESIA

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Abstract
Hepatitis B virus (HBV) infection is endemic in Indonesia. It causes infected people to suffer and imposes a tremendous burden on communities. Having high genome diversity, HBV is classified into ten genotypes—A to J—with distinct global geographical distributions. This article reviews HBV genotype distributions in the Wallacea region, the name given to the numerous islands and the surrounding seas east of Wallace's line, which is the world hotspot for marine species diversity. Genotype B (HBV/B) is predominant to the west of Wallace's line, and genotype C (HBV/C) is predominant to the east, in Wallacea itself. However, both of these genotypes, together with HBV/A and HBV/D and their numerous subgenotypes, have been identified in Wallacea. Five typical Indonesian HBV/B subgenotypes—B3, B5, B7, B8, and B9— prevail in Wallacea. Of 16 existing HBV/C subgenotypes, 13 are detected throughout Indonesia, with four—C1, C2, C5, C10—found in Wallacea. The high HBV diversity in Wallacea parallels the broad-scale pattern of marine species diversity. This region offers unique opportunities for collaborative studies to understand the adaptive mechanism of HBV in a variety of organisms.

***Key words*:** genetic diversity, genotype, hepatitis B virus (HBV), subgenotypes.

Introduction

Hepatitis B virus (HBV) infection is one of the major global public health problems. Approximately one third of the world's population have serological evidence of current or past HBV infection with around 240 million suffering from chronic hepatitis B. Of these, 75% reside in the Asia-Pacific

region including Indonesia. With 780,000 deaths per year due to the consequences of HBV infection that include hepatic failure, liver cirrhosis, and hepatocellular carcinoma (HCC), this disease has been recognized as a deadly threat (WHO, 2014). The World Cancer Report 2014 has stated that HCC is now the fifth most frequent cancer and the second leading cause of cancer deaths worldwide (Stewart and Wild, 2014).

Classification of the endemicity of HBV infection is based on the prevalence rate of the hepatitis B surface antigen (HBsAg), the serological marker of HBV infection. Uniquely among infectious diseases, the HBsAg prevalence is highly variable among populations of different continents and geographical regions. In sub-Saharan Africa, East Asia, Southeast Asia and Alaska, hepatitis B is highly endemic with HBsAg prevalence of >8%. Outside this region, the southern parts of Eastern and Central Europe, the Amazon basin, the Middle East and the Indian subcontinent are the regions of intermediate endemicity with HBsAg carriage of 2-8%, while southern and northern Europe, North America, and Australia are regions of low endemicity with HBsAg prevalence of <2%, (Lavanchy, 2004). In Indonesia, the prevalence of HBsAg varies in different areas with rates around 9.4% (3.4%-20.3%), classifying Indonesia as a moderate-to-high endemic region (Khan et al, 2004; NIHRD, 2010).

***Hepatitis B virus (HBV) genome diversity*:** HBV is a small partially double stranded DNA virus that belongs to the hepadnaviridae family. The DNA has 3,200 bp lengths and is divided into four overlapping Open Reading Frames (ORFs): Surface (S), Core (C), Polymerase (P) and X, which encode the surface (HBsAg), core and precore (HBcAg and HBeAg), polymerase and HBx proteins, respectively. The Surface gene is divided into three regions with three different stop codons: preS1, preS2, and S. Of these three regions, preS2 has the highest polymorphism within its sequence, while S has the lowest, which is recognized by the host immune system and used as the target of diagnosis and vaccine.

HBV replicates via reverse transcription from mRNA to DNA without having a proofreading mechanism. Therefore, high genome sequence diversity within the four genes is present. Ten HBV genotypes (A to J), which differ from each other by >8% of the entire genome (Kramvis *et al*., 2005; Schaefer, 2005), have been identified with genotypes B and C prevalent among Asians including Indonesian. Some genotypes have been classified into subgenotypes which differ from each other by 4-8% of the complete genome.

Notably, some genotypes have been shown to be associated with disease progression and response to treatment. In some studies, HBeAg seroconversion, which is considered to be a successful control of the virus, was found to be greater in patients infected with genotype B compared to those with genotype C (Orito *et al*., 2001; Ding *et al*., 2001). Other studies from Asia showed patients with genotype B were more likely to have sustained biochemical remission after spontaneous HBeAg seroconversion (Chu *et al*., 2002). Further, patients with genotype C showed less response to pegylated-interferon compared to those with genotype B (Cooksley *et al*., 2003).

Geography, ethnicity, and the Wallace's Line of Indonesia

Geographically, Indonesia is located at the cross point of three major global regions: Asia, Oceania, and Australia. There are some large islands such as Sumatra, Java, Bali, and Borneo in the west and the large island of New Guinea in the east, the western half of which covers the Indonesian provinces of Papua and Papua Barat. Wallace's Line separates the

ecozones of Asia and Wallacea, a transitional zone between Asia and Australia. The line runs through Indonesia between Borneo and Sulawesi, and through the Lombok straits between Bali and Lombok (Camerini, 2014). The region known as Wallacea is between this line and the island of New Guinea.

Indonesia is inhabited by more than 350 ethnic populations across the 17,000 islands of the entire archipelago (Sugiono, 2008). Ethnic groups are phenotypically different from each other. Different languages or dialects can easily be heard even within one ethnic population group. Overall, Indonesia has around 500 languages, including dialects (Sugiono, 2008). This high variety of ethnic populations and languages is found in the entire Indonesian archipelago. With the high endemicity of HBV infection and all the challenges posed by this disease, together with localised geographical distribution patterns and the high variety of ethnic populations, a greater understanding of HBV genome diversity in Indonesia is needed for the development of effective strategies for the prevention, control, and management of HBV infection. Reducing HBV-related morbidity and mortality, and thus, reducing the burden of HBV infection in Indonesia, is a high priority.

***Distribution of HBV genotype / subgenotype*:** HBV genotypes B (HBV/B) and C (HBV/C) are prevalent in Indonesia. HBV/B is predominant in populations of the islands to the west of the Wallace's Line (Sumatra, Java, Bali, and Borneo), while HBV/C is the major genotype in populations of Papua and its surrounding islands to the east of this line. However, both of these as well as two others, genotypes HBV/A and HBV/D, are found on islands within the Wallacea region (Lusida *et al*., 2008; Mulyanto *et al*., 2009; Thedja *et al*., 2011). HBV genotypes B, C, and D have been classified into several subgenotypes. Recently, HBV/B has been divided into nine subgenotypes: B1 to B9, while HBV/C has been divided into sixteen subgenotypes: C1 to C16. Each of the HBV/B subgenotypes is prevalent in different geographical parts of Asia: B1 (HBV/B1) in Japan, B2 (HBV/B2) in China, B3 (HBV/B3) in Indonesia, B4 (HBV/B4) in Vietnam, B5 (HBV/B5) in the Philippines, B6 (HBV/B6) in the Artic, and, B7 (HBV/B7), B8 (HBV/B8) and B9 (HBV/B9) in the eastern Nusa Tenggara islands of Indonesia (Norder *et al*., 1992; Norder *et al*., 2004; Sakamoto *et al*., 2006; Sakamoto *et al*., 2007; Nurainy *et al*., 2008; Mulyanto *et al*., 2009; Thedja *et al*., 2011). Thus, Indonesia, has six of the nine HBV/B subgenotypes and each has a distinct geographical distribution (Nurainy *et al*., 2008; Thedja *et al*., 2011). HBV/B3 is the major HBV/B subgenotype in Sumatra, Nias, Mentawai, Java, and Borneo, which are situated in the western half of Indonesia; and interestingly, HBV/B3 is also detected in small numbers in population of Lombok and Sulawesi islands of the eastern half of Indonesia. While HBV/B2 is the predominant HBV/B subgenotype found among Indonesian Chinese populations.

It is noteworthy that a variety of HBV/B subgenotypes are found within the Wallacea region. Five, B3, B5, B7, B8, and B9, prevail on Sulawesi, Lombok, and East Nusa Tenggara islands (Sumba, Flores, and Alor). HBV/B8 and B9 are found only in islands of East Nusa Tenggara, where they account for 7.5% (B8) and 30.6% (B9) (Thedja *et al*., 2011). A high number of HBV/B7 isolates was identified in East Nusa Tenggara islands in two studies, 13% (Mulyanto *et al*., 2009), and 43,5% (Thedja *et al*., 2011). However, this subgenotype was also detected in small numbers in the western half of Indonesia (Mulyanto *et al*., 2009; Thedja *et al*., 2011). These data demonstrate that HBV/B subgenotypes within the Wallacea region are much more heterogenous compared to those in western or far eastern parts of Indonesia. The existence of various HBV/B subgenotypes

in these areas is of interest and might be caused by inter- or intra-genotypic/subgenotypic recombination where some genotypes and subgenotypes are co-circulating and co-infecting the population. To better understand the formation and the existence of HBV high genetic diversity particularly in the Wallacea region, further study involving broad analysis of recombination events which might occur in S and/or Core regions of the virus would be necessary. Such understanding would help to develop an effective management strategy as well as a prevention strategy since HBV genetic divergence has been shown to play a role in the natural history of HBV infection (Huang *et al*., 2013).

Meanwhile, available data also show that thirteen (HBV/C1, C2, C5, C7, C8, C9, 10, C11, C12, C13, C14, C15, and C16) of the sixteen HBV/C subgenotypes are also found across the Indonesian archipelago, with the greatest diversity in Papua where 10, subgenotypes C7–C16, are found (Nurainy *et al*., 2008; Thedja *et al*., 2011; Mulyanto *et al*., 2012). A closer inspection of the Wallacea region reveals the presence of four subgenotypes (C1, C2, C5, and C8), with C5 found only in the northern islands of Sulawesi (Mulyanto *et al*., 2009; Thedja *et al*., 2011). The presence of numerous HBV/C subgenotypes demonstrates that Indonesia has a much greater HBV/C genome diversity than countries in the mainland of Asia that have more homogenous HBV/C genome characteristics, with only subgenotypes C1 and C2 present. A recent investigation found that the distribution of HBV/C subgenotypes, like those of HBV/B above, are geographically specific (Thedja *et al*., 2014), which might be explained by the patterns of human dispersal that occurred in the peopling of this archipelago thousands years ago (Thedja *et al*., 2011). In addition, two of six HBV/D subgenotypes, D1 and D3, have been found in the Wallacea region, as well as a small number of genotype A in this region and in the western half of Indonesia.

The specific ethnogeographical distribution of HBV genotypes has been assumed to be the result of selection by the host's immune response across generations (Jazayeri, 2005). The high number of HBV genomic types in the Wallacea region that parallels the vast wealth of marine species diversity could be attributable to the hybridization of two biogeographical zones, and may place this region as a Biodiversity Hotspot where parallel evolution and genotypic adaptation have occurred in organisms ranging from microbes to plants, invertebrates, vertebrates and primates. This region provides a rare opportunity to compile a wealth of data connecting genotype to phenotype, allowing researchers to identify and compare the genetic mechanisms underlying adaptive traits in a variety of organisms.

Conclusion

In conclusion, a high genetic diversity of HBV is observed in the Wallacea region of the Indonesian archipelago, with the predominance of genotypes B and C and their numerous subgenotypes. Genotypes A and D are also present as minor genotypes in this region. The data on the distribution patterns of various genotypes and subgenotypes parallels the broad-scale patterns of marine species diversity to the east of Wallace's Line, providing a potential field laboratory for the study of evolution. As the outcome of HBV infection (that ranges from asymptomatic carrier state to progressive diseases that include chronic active hepatitis, liver cirrhosis, and hepatocellular carcinoma) is determined by host and virus interaction with the influence of environmental factors, this huge biodiversity is of significance to understand the pathomechanisms of HBV-related diseases. Collaborative studies involving many related fields, such as biomedical, biochemical, and biophysical sciences are needed to allow researchers to

identify and compare the mechanisms underlying adaptive traits in a variety of organisms, providing the basis to understand disease processes and develop strategies and clues to overcome problems faced by humans.

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