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Hepatitis B Virus Infection in Indonesia 15 Years after Adoption of a Universal Infant Vaccination Program: Possible Impacts of Low Birth Dose Coverage and a Vaccine-Escape Mutant

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Abstract. A universal hepatitis B vaccination program for infants was adopted in Indonesia in 1997. Before its implementation, the prevalence of hepatitis B surface antigen (HBsAg)–positive individuals in the general population was approximately 5–10%. The study aimed to investigate the hepatitis B virus (HBV) serological status and molecular profile among children, 15 years after adoption of a universal infant vaccination program in Indonesia. According to the Local Health Office data in five areas, the percentages of children receiving three doses of hepatitis B vaccine are high (73.9–94.1%), whereas the birth dose coverage is less than 50%. Among 967 children in those areas, the seropositive rate of HBsAg in preschool- and school-aged children ranged from 2.1% to 4.2% and 0% to 5.9%, respectively. Of the 61 HBV DNA–positive samples, the predominant genotype/subtype was B/*adw2*. Subtype *adw3* was identified in genotype C for the first time in this population. Six samples (11.5%) had an amino acid substitution within the *a* determinant of the *S* gene region, and one sample had T140I that was suggested as a vaccine-escape mutant type. The low birth dose coverage and the presence of a vaccine-escape mutant might contribute to the endemicity of HBV infection among children in Indonesia.

INTRODUCTION

Indonesia experiences intermediate to high hepatitis B virus (HBV) endemicity. Before adoption of universal infant vaccination, the prevalence of hepatitis B surface antigen (HBsAg) seropositivity was estimated to be 5–10% in the general population.[1,2] The perinatal transmission route has played an important role in the increasing number of infants who have become infected with HBV.[3]

A universal hepatitis B vaccination program for infants was adopted in Indonesia in 1997. The HepB3 coverage (defined as the percentage of children receiving three doses of hepatitis B vaccine) in 2011 was estimated by the World Health Organization to be 63%; however, this number varies in each area in Indonesia.[4,5]

The widely distributed hepatitis B vaccine consists of recombinant HBsAg, which has a major antibody-neutralizing epitope region, termed the "*a* determinant." Amino acid changes within this region could make HBV resistant to the neutralizing effect of anti-HBsAg (anti-HBs) and could threaten the effectiveness of the hepatitis B vaccination program.[6] Moreover, the emergence of HBV variants with mutations within the small surface protein (S) after the implementation of the hepatitis B universal vaccination program has increased through the years and may cause vaccination failure.[7,8]

Nine HBV genotypes, from A to H and J, that are based on its surface gene, have been documented,[9–12] and HBV genotypes B and C were predominant among Indonesian populations.[13–16] Ten HBV serological subtypes, which are based on the amino acid sequences of HBsAg, have been identified.[17–19] Indonesia has been geographically divided into four different HBV subtype zones: the *adw*, *adr*, *ayw*, and *mix* zones.[20] It is important to record the *S* gene variant type in an endemic population because this information may be critical for determining policies for vaccine and diagnostic reagent design.[21–23]

Fifteen years after the implementation of the universal vaccination program, the serological status and molecular profile of HBV in children has still not been fully investigated. This study aimed to analyze the prevalence and significance of HBV variants, including emerging vaccine-escape mutants, among children in several districts in Indonesia based on HBV subtype and genotype zones.

MATERIALS AND METHODS

Study subjects. Serum samples were collected from preschool-aged (1–5 years) and school-aged (6–12 years) children during April–October 2012. The following four cities were included in this study: Kotawaringin Barat in Central Kalimantan—*adw*/*mix* zone (N = 258), Kupang in West Timor—*ayw* zone (N = 177), Sorong in West Papua—*adr* zone (N = 167), and Bau-bau in southeast Sulawesi—*mix* zone (N = 136). The data for Lamongan, East Java, which is an *adw* zone (N = 229), were obtained from a previous study conducted in 2007.[14] Serum samples were sent to the Institute of Tropical Disease, Airlangga University, Surabaya, Indonesia.

Written informed consent was obtained from the parents of the children. However, some of the individual vaccination records were not available. The hepatitis B immunization coverage data of each area were provided by the Local Health Office. The study protocol was reviewed and approved by the ethics committees of Kobe University in Japan and Airlangga University in Indonesia.

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Serological markers of HBV infection. All refrigerated samples were tested for HBsAg and anti-HBs using enzyme-linked immunoabsorbant assays (ELISAs) (Hepalisa HBsAg and New Hepalisa Anti HBs kits, respectively; Indec Diagnostic, Jakarta, Indonesia). To differentiate vaccine-induced antibody from natural infection-acquired antibody, the antibody to HBV core antigen (anti-HBc) was examined by ELISA (Hepalisa Anti HBc; Indec Diagnostic).

Molecular detection of HBV DNA. All HBsAg-positive (N = 24) and HBsAg-negative/anti-HBc-positive (N = 79) samples were examined for HBV DNA to confirm infection and identify the surface antigen. DNA was extracted from 200 μL of serum using a DNA extractor kit (QIAamp DNA Blood Mini Kit; QIAGEN, Tokyo, Japan). The presence of HBV DNA was then assessed by nested polymerase chain reaction (PCR) assays using primers P7 and P8, which target nucleotides 256–796 of the *S* gene, as the first primer pair. When the first PCR amplification was negative, a second round of PCR was carried out using primers HBS1 and HBS2, which target nucleotides 455–713 of the *S* gene. The amplification products were visualized on a 2% agarose gel stained with ethidium bromide.[13]

Nucleotide sequence analysis. Nucleotide sequencing of the amplified fragments was performed using the BigDye deoxy Terminator v1.1 cycle sequencing kit with an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA), as described previously,[13] to determine the HBV genotype and subtype and the possible presence of another HBV *S* gene variant (vaccine-escape mutant). HBV genotypes were determined based on homology (> 96%) to the *S* gene[17,24] using Genetyx Win v9.0 (Genetyx Corporation, Tokyo, Japan). HBV nucleotide sequences were aligned using the Bioedit program and were analyzed further. HBV subtypes were then deduced on the basis of the predicted amino acid sequence substitution at positions 122, 127, 134, 140, 159, 160, and 177 of the *S* gene, as previously described.[9,10,19,25] Other amino acid substitutions in the *S* gene related to the vaccine-escape mutant were also analyzed.

RESULTS

The subjects from East Java and South East Sulawesi included school-aged children who ranged in age from 8 to 13 years. The subjects from Central Kalimantan and West Timor included both preschool- and school-aged children. The subjects from West Papua included only preschool-aged children (Table 1).

The seropositive rates of HBsAg among the preschool-aged children in the five areas ranged from 2.1% to 4.2%, and those among school-aged children ranged from 0% to 5.9%. The prevalence of anti-HBs seropositivity among preschool-aged children was higher (61.4–65.8%) than among school-aged children (20.9–40.4%). The prevalence of anti-HBc seropositivity among preschool- and school-aged children ranged from 3.5% to 4.8% and from 5.2% to 50.4%, respectively. The highest prevalence of anti-HBc seropositivity was found in southeast Sulawesi.

In this study, HBV DNA was detected in 62 samples with either HBsAg positivity or HBsAg negativity/anti-HBc positivity from five areas (Table 1). The HBV genotypes that were found among children in Indonesia included genotype B (68.8%) and C (31.2%). Genotype B was predominant in East Java (90.9%), Central Kalimantan (100%), and southeast Sulawesi (60%), whereas both genotypes B and C were common in West Timor and West Papua (Table 2).

The HBV subtype prevalence of each area also varied. The HBV subtype *adw* was predominant among children in East Java and Central Kalimantan (90.9% and 100%, respectively) (Table 2). Meanwhile, subtype *adr* was predominant among children in West Papua, and two samples (PB 09 and PB 26) had the A159/A177 amino acid combination and were classified as *adrq* indeterminate[13] (Figure 1). In southeast Sulawesi, subtypes *ayw* and *adr* were commonly found among school-aged children (44.0% and 40%, respectively). In West Timor, HBV subtypes *ayw* and *adw* were common in prevalence, 33.3% and 44.4%, respectively, and subtype *adw3* was identified for the first time in Indonesia.

In this study of 32 samples with genotype B, the number of samples identified as subtypes *adw2* or *ayw* was 28 and 14, respectively. HBV genotype C with subtype *adrq+* was predominant in Indonesia and was found in 15 of 19 samples. Other subtypes identified in HBV genotype C included *adw2*, *adw3*, and *adrq(i)*.

Of the 61 HBV DNA–positive samples, six (11.5%) had an amino acid substitution within the *a* determinant of the *S* gene region (Table 3); all of these samples were genotype B and subtype *adw2*. Four samples were found among preschool-aged children (PB53, PB54, PB173, and NT43). However, the anti-HBc (PB173) status of one isolate with insufficient serum could not be determined. Two types

TABLE 1
Characteristics of subjects and seropositive prevalence among preschool- and school-aged children in several areas in Indonesia*

Characteristic of subjects	Preschool-aged children			School-aged children			
	Central Kalimantan	West Timor	West Papua	Central Kalimantan	West Timor	southeast Sulawesi	East Java (prior study, 2007)[14]
No. of subjects (male, female)	143 (77, 66)	73 (34, 39)	167 (89, 78)	115 (64, 51)	104 (48, 56)	136 (85, 51)	229 (113, 116)
Mean age ± SD (years)	2.7 ± 1.3	4.2 ± 0.9	2.9 ± 1.4	9.9 ± 1.5	9.1 ± 1.8	10.5 ± 0.7	10.5 ± 1.1
Birth dose HB vaccination coverage (%)†	40.9	46.8	24.8	No data	No data	No data	No data
DPT-HB3 vaccination coverage (%)†‡	94.1	73.9	91.0	No data	No data	62.8	90.0
No. of HBsAg positive (%)	3 (2.1)	2 (2.7)	7 (4.2)	0 (0)	4 (3.8)	8 (5.9)	7 (3.1)
No. of anti-HBs positive (%)	94 (65.7)	48 (65.8)	102 (61.4)	24 (20.9)	42 (40.4)	47 (34.6)	54 (23.6)
No. of anti-HBc positive (%)	5 (3.5)	3 (4.1)	8 (4.8)	6 (5.2)	6 (5.8)	68 (50.4)	50 (23.8)
Detectable HBV DNA§	5/6	3/4	8/11	3/6	6/7	25/68	12/55

HB = hepatitis B; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus; SD = standard deviation.
*Preschool- and school-aged children groups were described as 1–5 and 6–12 years, respectively.
†The data were obtained from the Local Health Office in each area.
‡DPT-HB3: complete dose of hepatitis B vaccine (three doses vaccines of combined diphtheria, pertussis, and tetanus toxoid–Hepatitis B).
§Detection of HBV DNA was performed for HBsAg-positive and/or anti-HBc-positive samples.

TABLE 2
Distribution of HBV genotype and subtype among children

HBV genotypes/subtypes	Western part		Eastern part			Total
	East Java* (prior study, 2007)[14]	Central Kalimantan	southeast Sulawesi	West Timor	West Papua	
HBV/B						
adw2	10 (90.9%)	8 (100%)	4 (16.0%)	2 (22.2%)	4 (50.0%)	28 (45.9%)
ayw1	–	–	11 (44.0%)	3 (33.3%)	–	14 (22.8%)
HBV/C						
adw2	–	–	–	1 (11.1%)	–	1 (1.6%)
adw3	–	–	–	1 (11.1%)	–	1 (1.6%)
adrq+	1 (9.1%)	–	10 (40.0%)	2 (22.2%)	2 (25.0%)	15 (24.6%)
adrq(i)	–	–	–	–	2 (25.0%)	2 (3.2%)
	11	8	25	9	8	61

HBV = hepatitis B virus.
*Of 12 samples with HBV DNA–positive from East Java, one sample was undetermined for HBV genotype and subtype.[14]

of single mutations were identified (T140I and S155F), and others had mixed mutations within the *a* determinant (P120S-A159V and I152T-F158L). Another sample had mixed mutations in both the inner and outer regions of the protein (M133L and S171F).

Three samples were HBV DNA positive but HBsAg negative, suggesting they resulted from occult hepatitis B infections (sample numbers NT43, KM61, and SS112). One of these isolates, NT43, which was anti-HBs positive, had a threonine-to-isoleucine amino acid substitution at position 140 (Figure 1).

DISCUSSION

Indonesia adopted a universal hepatitis B vaccination program for infants in 1997. Plasma-derived vaccine was produced until 1997, when it was replaced by a recombinant hepatitis B vaccine.[2,14] In 1999, Indonesia introduced an innovative policy by using a prefilled single-use injection device of hepatitis B vaccine that is stable even outside the cold chain. This approach has improved hepatitis B vaccine coverage and reduced hepatitis B carrier rates.[26] In 2000, Indonesia introduced a program for using the device for the birth dose in seven provinces. In 2003, the program expanded to target all of Indonesia's 5 million annual births.[27]

The ultimate goal of the national hepatitis B immunization program is an HBsAg prevalence of less than 1% in 5-year-olds born after the start of routine immunization.[28] This study revealed that the prevalence of HBsAg among preschool- and school-aged children ranged from 2.1% to 4.2% and 0% to 5.9%, respectively (Table 1). These rates were lower compared with a previous study on adult (from 5 to 10%) and pregnant female (5.2%) populations in Indonesia.[2] According to the first immunization project model of hepatitis B vaccination among children in Lombok in 1987–1991, the carriage rates decreased from 6.2% to 1.4%.[29] In Taiwan,

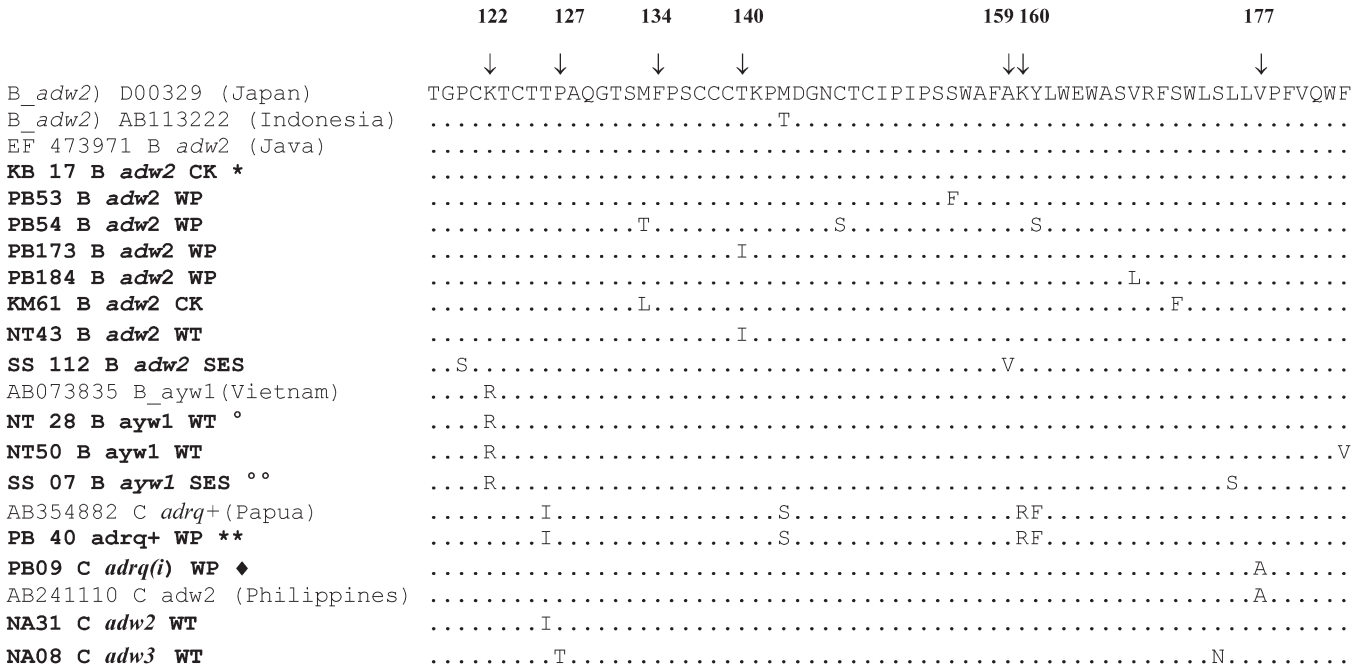

FIGURE 1. Multiple alignment of the amino acid sequences of hepatitis B surface antigen (positions 118–183) of hepatitis B virus isolates among children in Indonesia (shown in bold) and of those from the international DNA databank (indicated with the accession number and origin), ↓ residues that determine subtype. * = 10 others; ° = 1 another; ** = 13 others; °° = 10 others; ♦ = 1 another.

TABLE 3
Characteristics of HBV DNA–positive subjects with an amino acid mutation within the *a* determinant region of HBsAg

Subject ID	Age (year)/sex	Area/ethnic group	Genotype/subtype	HBsAg	Anti-HBs	Anti-HBc	Amino acid mutation*
PB53	2/M	WP/Sulawesi	B/*adw2*	+	+	−	S155F
PB54	4/F	WP/Sulawesi	B/*adw2*	+	+	−	M133T, C147S
PB173	4/M	WP/Papua	B/*adw2*	+	−	Not tested	T140I
NT43	4/F	WT/Timor	B/*adw2*	−	+	+	T140I
KM61	12/M	CK/Malay	B/*adw2*	−	−	+	M133L
SS112	10/F	SES/Sulawesi	B/*adw2*	−	−	+	P120S; A159V

CK = Central Kalimantan; F = female; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus; M = male; SES = southeast Sulawesi; WP = West Papua; WT = West Timor.
*Amino acid substitutions within the *a* determinant region (aa 100–160).

one of the highest hepatitis B–endemic countries to successfully implement a universal vaccination program, the prevalence of HBsAg decreased from 9.8% in 1984, the year when universal infant immunization began, to 0.7% in 1999, 15 years later.[30,31]

Hepatitis B vaccines and immunoglobulin (HBIG) are highly effective methods (89–98%) of preventing perinatal transmission if given within 12–24 hours after birth.[32–35] According to the national immunization policy in Indonesia, the birth dose of the hepatitis B vaccine should be given within 7 days after birth and should be followed by three doses of combination vaccines including diphtheria, pertussis, tetanus and hepatitis B within the 2nd, 3rd, and 4th months (DPT-HB3).[27,36] The parents should bring their baby to the primary health center to receive the vaccine; however, many people in remote areas in Indonesia have difficulty reaching these centers due to geographical isolation.[3] Furthermore, screening for hepatitis B to all pregnant women is not implemented as a national program in Indonesia,[37] and most infants born to women with hepatitis B would therefore not have received HBIG. If the mother is infected and transmits the virus before the child is vaccinated, and no HBIG is given simultaneously within 24 hours of birth, vaccination would not protect the child.[4]

According to the Local Health Office data, DPT-HB3 coverage in Central Kalimantan, West Timor, and West Papua is quite high (73.9–94.1%) (Table 1). In contrast, the birth dose coverage of the hepatitis B vaccine in those areas is less than 50%. Many people in Indonesia who live far from health service centers prefer giving birth at home and finding a traditional birth attendant to deliver the baby.[3] These facts could delay the scheduling of the birth dose of hepatitis B vaccine.

In this study, anti-HBs seropositivity prevalence among preschool-aged children was higher (61.4–65.8%), than among school-aged children (20.9–40.4%) (Table 1). A previous study among vaccinated children in Taiwan had shown that the anti-HBs positivity rates for 2-year-old children were as high as 93–97%.[36] The antibody titer then gradually fell to less than 10 mIU/mL by 10–15 years.[38,39] The results of this study may reflect a similar decline in anti-HBs levels; however, the lower immunization coverage in Indonesia during earlier years of its implementation (before 2003)[27] may also have contributed to these results.

This study demonstrated the current geographical distribution pattern of the HBV genotypes and subtypes among children 15 years after implementation of the universal vaccination program. A previous study identified four genotypes (A, B, C, and D) among adults in Indonesia; however, genotypes B and C showed predominant distributions.[15] Furthermore, the four zones of HBV subtypes shared the pattern that was identified in Indonesia: *adw*, *ayw*, *adr*, and *mix* zones.[20]

This study found that genotype B and subtype *adw* were predominant in western Indonesia, that is, East Java and Central Kalimantan (Table 2). There were no altered genotype or subtype patterns in Java, where most ancestors of the people migrated from Indo-Chinese areas where *adw* predominates. Subtype *adw* was also the main subtype in Central Kalimantan, possibly because the ancient people of Kotawaringin Barat mostly originated from Sumatra and Java Island.

Both genotypes B and C were significantly found in regions of eastern Indonesia, that is, southeast Sulawesi, West Timor, and West Papua. Genotype B mostly originated from China, Japan, and southeast Asia.[18] Genotype C mainly originated from Far East Asia, Australia, New Zealand, and Polynesia, and it was predominant in eastern Indonesia, including North Sulawesi, Moluccas, and Papua.[13,15,16] Both areas in southeast Sulawesi and West Timor showed a mixed pattern of HBV subtypes. On the other hand, there was an altered pattern of the main subtype in West Papua, from *adr* to both *adr* and *adw* subtypes. Currently, the demographical data reveal that many citizens who live in West Papua migrated from outside the island, mostly from Java, Sulawesi, and Moluccas.[38]

This study found that genotype B and subtype *adw* were common among children in all areas of Indonesia. The genotype and subtype geographical distribution pattern findings in this study are a result of various ethnic groups living in Indonesia and of rapid interisland population movements within the last decades.

HBV subtype *adw3*, which was previously published as a new subtype,[39] was identified in one sample (NA 08) from West Timor and was also determined to be a new subtype in this study. It has been known that amino acid positions 122 and 160 are responsible for the expression of *d/y* and *w/r* specificity[40]; in addition, the presence of threonine at position 127 is necessary to distinguish *w3* specificity.[41] Interestingly, subtype *adw3*, which belonged to genotype C but had been officially recognized in genotype B and D, was first identified in Indonesia.[19,39]

The major target of the neutralizing antibody produced by natural infection or vaccination is the *a* determinant within HBsAg.[42] The *a* determinant region represents the immunodominant HBsAg, which was initially considered as a conformational cluster of epitopes located between residues 120 and 150 of HBsAg and has currently been expanded to residues 110–160 after the discovery of two more epitopes.[43] Alteration of residues within the *a* determinant region can result in reduced antigenicity and protein expression, which can result in failure to neutralize viral infection and can

affect diagnostic assay detection.[2,44,45] The previous study in East Java suggested T126I substitution, in combination with T143I, in genotype B could affect the antigenicity of HBsAg.[14] The most common and widely recognized HBV vaccine-escape mutant type is the one with a change at position 145, from glycine to arginine.[45,46] The G145R mutation was not found in this study and has not yet been reported in Indonesia.

Three samples were HBV DNA positive and anti-HBs positive, which had an amino acid mutation within the *a* determinant region (PB53, PB 54, and NT43). One of these samples, NT43, had a T140I amino acid substitution, which has also been potentially suggested as a vaccine-escape mutant type. In contrast, this mutation was also previously found in unvaccinated Taiwanese children.[47] Several alterations in the *a* determinant region (positions 121–149), particularly those located within the highly hydrophilic residues 137–149 of the major HBsAg epitope, are known as vaccine-escape mutations during immunization.[48] The amino acid substitution M133T, which was detected in sample PB54, was also previously reported in an adult patient with chronic liver disease in Korea.[49]

The M133L mutation that was identified in this study, sample KM 61, was also previously reported in occult HBV infection among adult blood donors and vaccinated children in Indonesia.[50,51] This mutation showed the least significant changes in antigenicity profile (compared with T143M/L, T123N, and M133IT/V), which may have slightly decreased the sensitivity of the HBsAg assay.[52,53] Other mutation types in samples PB 53 and SS 112, that is, S155F and P120S-A159V, respectively, should be studied further in the future to determine their association with vaccination failure.

Limitations to this study included the following: some of the individual vaccination records were not available, and the vaccination coverage rate used in this study was based on the Local Health Office data from a year before sample collection. Although our sampling strategy was nonrandom, our results from five representative areas might nonetheless describe the general situation of hepatitis B infection among children based on the HBV subtype zones in Indonesia.[20]

In conclusion, this study finds that HBsAg seropositivity among populations of children in Indonesia has decreased but HBV still remains low to moderately endemic 15 years after adoption of a universal infant vaccination program. The low birth dose coverage of hepatitis B vaccination and the presence of an HBV vaccine-escape mutant may have contributed to these findings. Altered HBV genotype and subtype distribution patterns were observed in several areas, and the migration of populations is suggested as one of the associated factors. Further studies with larger-scale subject populations and complete individual vaccination records are necessary to obtain empirical data for evaluating the hepatitis B vaccination program in Indonesia.

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REFERENCES

1. Suwignyo S, 1996. Seroepidemiology of hepatitis B and C in Indonesia. *Int Hepatol Commun 5:* 30–37.
2. Khan M, Dong JJ, Acharya SK, Dhagwardorj Y, Abbas Z, Jafri W, Mulyono DH, Tozun N, Sarin SK, 2004. Hepatology issues in Asia: perspective from regional leaders. *J Gastroenterol Hepatol 19:* S419–S430.
3. Creati M, Saleh A, Ruff TA, Stewart T, Otto B, Sutanto A, Clements CJ, 2007. Implementing the birth dose of hepatitis B vaccine in rural Indonesia. *Vaccine 25:* 5985–5993.
4. World Health Organization, Regional Office for southeast Asia, 2013. *Regional Strategy for the Prevention and Control of Viral Hepatitis*. Available at: http://www.searo.who.int/entity/emerging_diseases/topics/CD_282.pdf. Accessed March 16, 2015.
5. National Institute of Health Research and Development, Ministry of Health of the Republic Indonesia, 2008. *Report on National Basic Health Research 2007*. Available at: http://terbitan.litbang.depkes.go.id/penerbitan/index.php/blp/catalog/book/22. Accessed August 10, 2013.
6. Carman WF, 1997. The clinical significance of surface antigen variants of hepatitis B virus. *J Viral Hep 4:* 11S–20S.
7. Yamamoto K, Horikita M, Tsuda F, Itoh K, Akahane Y, Yotsumoto S, Okamoto H, Mikakawa Y, Mayumi M, 1994. Naturally occurring escape mutants of hepatitis B virus with various mutations in the *S* gene in carriers seropositive for antibody to hepatitis B surface antigen. *J Virol 68:* 2671–2676.
8. Hsu HY, Chang MH, Ni YH, Chen HL, 2004. Survey of hepatitis B surface variant infection in children 15 years after a nationwide vaccination programe in Taiwan. *Gut 53:* 1499–1503.
9. Okamoto H, Tsuda F, Sakugawa H, Satrosoewignjo RI, Imai M, Miyakawa Y, Mayumi M, 1988. Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. *J Gen Virol 69:* 2575–2583.
10. Kramvis A, Kew M, Francois G, 2005. Hepatitis B genotypes. *Vaccine 23:* 2409–2423.
11. Seeger C, Zoulim F, Mason WS, 2013. Hepadnaviruses. Knipe DM, Howley PM, eds. *Fields Virology*, 6th edition. Philadelphia, PA: Lippincott, Williams and Wilkins, 2185–2221.
12. Tatematsu K, Tanaka Y, Kurbanov F, Sagauchi F, Mano S, Maeshiro T, Nakayoshi T, Wakuta M, Miyakawa Y, Mizokami M, 2009. A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. *J Virol 83:* 10538–10547.
13. Lusida MI, Nugrahaputra VE, Soetjipto, Handayani R, Naganofujii M, Sasayama M, Utsumi T, Hotta H, 2008. Novel subgentypes of hepatitis B virus genotype C and D in Papua, Indonesia. *J Clin Microbiol 46:* 2160–2166.

14. Utsumi T, Yano Y, Lusida MI, Amin M, Soetjipto, Hotta H, Hayashi Y, 2010. Serologic and molecular characteristics of hepatitis B virus among school children in East Java, Indonesia. *Am J Trop Med Hyg 83:* 189–193.
15. Mulyanto, Depamede SN, Surayah K, Tsuda F, Ichiyama K, Takahashi M, Okamoto H, 2009. A nationwide molecular epidemiological study on hepatitis B virus in Indonesia: identification of two novel subgenotypes, B8, C7. *Arch Virol 154:* 1047–1059.
16. Juniastuti, Lusida MI, Nugrahaputra VE, Amin M, Utsumi T, Hayashi Y, Hotta H, 2011. Another novel subgenotype of hepatitis B virus genotype C from Papuans of Highland origin. *J Med Virol 83:* 225–234.
17. Magnius LO, Norder H, 1995. Subtype, genotype and molecular epidemiology of the hepatitis B virus as reflected by sequence variability of the *S* gene. *Intervirology 38:* 24–34.
18. Norder H, Courouce AM, Coursaget P, Echevaria JM, Lee SD, Mushahwar IK, Robertson BH, Locarnini S, Magnius LO, 2004. Genetic diversity of hepatitis B virus strains derived worldwide: genotype, subgenotypes, HBsAg subtypes. *Intervirology 47:* 289–309.
19. Purdy MA, Talekar G, Swenson P, Araujo A, Fields H, 2007. A new algorithm for deduction of hepatitis B surface antigen subtype determinants from the amino acid sequence. *Intervirology 50:* 45–51.
20. Mulyanto, Tsuda F, Karossi AT, Soewignyo S, Roestamsjah, Sumarsidi D, Trisnamurti RH, Sumardi, Surayah, Udin LZ, Wikanta M, Kanai K, Mishiro S, 1997. Distribution of the hepatitis B virus in Indonesia: implication for heterogeneity and infection control measures. *Arch Virol 142:* 2121–2129.
21. Zuckerman AJ, Zuckeman JN, 1999. Molecular epidemiology of hepatitis B virus mutant. *J Med Virol 58:* 193–195.
22. Zuckerman AJ, 2000. Effect of hepatitis B virus mutants on efficacy of vaccination. *Lancet 355:* 1382–1384.
23. Francois G, Kew M, van Damme P, Mphahlele MJ, Mehews A, 2001. Mutant hepatitis B viruses: a matter of academic interest only or a problem with far reaching implications? *Vaccine 19:* 3799–3815.
24. Arauz-Ruiz P, Norder H, Visona KA, Magnius LO, 1997. Molecular epidemiology of hepatitis B virus in Central America reflected in the genetic variability of the small *S* gene. *J Infect Dis 176:* 851–858.
25. Norder H, Courouce AM, Magnius LO, 1994. Complete genome, phylogenetic relatedness and structural protein of six strains of hepatitis B virus, four of which represent two new genotypes. *Virology 198:* 489–503.
26. Nelson CM, Wibisono H, Purwanto H, Mansyur I, Moniaga V, Widjaya A, 2004. hepatitis B vaccine freezing in Indonesia cold chain: evidence and solutions. *Bull World Health Organ 82:* 99–105.
27. Levin CE, Nelson CM, Widjaya A, Moniaga V, Anwar C, 2004. The costs of home delivery of a birth dose of hepatitis B vaccine in prefilled syringe in Indonesia. *Bull World Health Organ 83:* 456–461.
28. Clements CJ, Baoping Y, Crouch A, Hipgrave D, Mansoor O, Nelson CB, Treleaven S, Konkelenberg RV, Wirsma S, 2006. Progress in the control of hepatitis B infection in the Western Pacific Region. *Vaccine 24:* 1975–1982.
29. Ruff TA, Gertig D, Otto BF, Gust ID, Sutanto A, Soewarso TI, Kandun N, Marschner IC, Maynard JE, 1995. Lombok hepatitis B model immunization project: toward universal infant hepatitis B immunization in Indonesia. *J Infect Dis 171:* 290–296.
30. Chan CY, Lee SD, Lo KJ, 2004. Legend of hepatitis B vaccination: the Taiwan experience. *J Gastroenterol Hepatol 19:* 121–126.
31. Chen DS, 2009. Hepatitis B vaccination: the key towards elimination and eradication of Hepatitis B. *J Hepatol 50:* 805–816.
32. Hipgrave DB, Maynard JE, Beverley AB, 2006. Improving birth dose coverage of hepatitis B vaccine. *Bull World Health Organ 84:* 65–71.
33. Greenberg DP, 1993. Pediatric experience with recombinant hepatitis B vaccine in infants and relevant safety and immunogenicity studies. *Pediatr Infect Dis J 12:* 438–445.
34. Otto BF, Suarnawa IM, Stewart T, Nelson C, Ruff TA, Widjaya A, Maynard JE, 2000. At birth immunization against hepatitis B using novel pre-filled immunization device stored outside the cold chain. *Vaccine 18:* 498–502.
35. Cui F, Li L, Hadler SC, Wang F, Zheng H, Chen Y, Gong X, Hutin YJ, Cairns KL, Liang X, Yang W, 2010. Factors associated with effectiveness of the first dose of vaccine in China: 1992–2005. *Vaccine 28:* 5973–5978.
36. Ministry of Health of the Republic Indonesia, 2004. *National Guideline of Vaccination Implementation*. Available at: http://www.hukor.depkes.go.id. Accessed August 20, 2012.
37. World Health Organization, 2013. *Global Policy Report on the Prevention and Control of Viral Hepatitis*. Available at: http://www.who.int/csr/disease/hepatitis/global_report/en/. Accessed March 16, 2015.
38. National Center for Statistics of the Republic Indonesia, 2010. *Life Time Migration: 1971–2010*. Available at: http://www.bps.go.id/linkTableDinamis/view/id/855. Accessed October 27, 2015.
39. Arauz-Ruiz P, Norder H, Robertson BH, Magnius LO, 2002. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. *J Gen Virol 83:* 2059–2073.
40. Okamoto H, Imai M, Tsuda F, Tanaka T, Miyakawa Y, Mayumi M, 1987. Point mutation in the S gene of hepatitis B virus for a d/y or w/r subtypic change in the two blood donors carrying a surface antigen of compound subtype adyr or adwr. *J Virol 61:* 3030–3034.
41. Norder H, Courouce AM, Magnius LO, 1992. Molecular basis of hepatitis B virus serotype variations within the four major subtypes. *J Gen Virol 73:* 3141–3145.
42. Waters JA, O'Rourke SM, Richardson SC, Papavengelou G, Thomas HC, 1987. Quantitative analysis of the humoral immune response to the "a" determinant of HBs antigen after inoculation with plasma derived or recombinant vaccine. *J Med Virol 21:* 155–160.
43. Carman WF, Van Deursen FJ, Mimms LT, Hardie D, Coppola R, Decker R, Sanders R, 1997. The prevalence of surface antigen variants of hepatitis B virus in Papua New Guinea, South Africa and Sardinia. *Hepatology 26:* 1658–1666.
44. Carman WF, Zanetti AR, Karayiannis P, Waters J, Manzillo G, Tanzi E, Zuckerman AJ, Thomas HC, 1990. Vaccine induced escape mutant of hepatitis B virus. *Lancet 336:* 325–329.
45. Coleman PF, 2006. Detecting hepatitis B surface antigen mutants. *Emerg Infect Dis 12:* 198–203.
46. Horvat RT, 2011. Diagnostic and clinical relevance of HBV mutations. *LabMedicine 42:* 488–496.
47. Hsu HY, Chang MH, Liaw SH, Ni YH, Chen HL, 1999. Changes of hepatitis B surface antigen variants in carrier children before and after vaccination in Taiwan. *Hepatology 30:* 1312–1317.
48. Gerin JL, Alexander H, Shih JW, Purcell RH, Dapolito G, Engle R, Green N, Sutcliffe JG, Shinnick TM, Lerner RA, 1983. Chemically synthesized peptides of hepatitis B surface antigen duplicate the d/y specificities and induced subtype-specific antibodies in chimpanzees. *Proc Natl Acad Sci USA 80:* 2365–2369.
49. Song BC, Kim SH, Kim H, Ying YH, Kim HJ, Kim YJ, Yoon JH, Lee HS, Cha CY, Kook YH, Kim BJ, 2005. Prevalence of naturally occurring surface antigen variants of hepatitis B virus in Korea patients infected chronically. *J Med Virol 76:* 194–202.
50. Thedja MD, Roni M, Harahap AR, Siregar NC, Ie SI, Muljono DH, 2010. Occult hepatitis B in blood donors in Indonesia: altered antigenicity of the hepatitis B virus surface protein. *Hepatol Int 4:* 608–614.
51. Utsumi T, Lusida MI, Yano Y, Purwono PB, Amin M, Soetjipto, Hotta H, Hayashi Y, 2014. Progress in the control of hepatitis B virus infection among children in Indonesia. *J Vaccines Vaccin 5:* 247.
52. Ie SI, Thedja MD, Roni M, Muljono DH, 2010. Prediction of conformational changes by single mutation in the hepatitis B virus surface antigen (HBsAg) identified in HBsAg-negative blood donors. *Virol J 7:* 326.
53. Scheiblauer H, El Nageh M, Diaz S, Nick S, Zeichhardt H, Grunert HP, Prince A, 2010. Performance evaluation of 70 hepatitis B virus (HBV) surface antigen (HBsAg) assays from around the world by a geographically diverse panel with an array of HBV genotypes and HBsAg subtypes. *Vox Sang 98:* 403–414.