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Protectivity and safety following recombinant hepatitis B vaccine with different source of bulk compared to hepatitis B (Bio Farma) vaccine in Indonesia

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Purpose: Indonesia, a high populous and the second-highest country in epidemicity of hepatitis B in South-East Asia require maintaining its capacity of monovalent hepatitis B production to keep up with both the national immunization program and global needs. To keep the sustainability of the vaccine, a new bulk is needed to be made available. This study aims to evaluate the immunogenicity and safety of Bio Farma newly formulated recombinant hepatitis B vaccines, which came from different sources of bulk, compared to the already registered hepatitis B vaccine.

Materials and Methods: An experimental, randomized, double-blind, cohort intervention phase II clinical trial was conducted on three recombinant hepatitis B vaccines from different bulk sources, with Bio Farma registered hepatitis B vaccine as the control group. A total of 536 participants around age 10 to 40 years old were thricely vaccinated with twice serological assessments. The subject's safety was monitored for 28 days after each vaccination.

Results: Of 536 enrolled participants, 521 finished the vaccination and serology assessments. The investigational products were proven not to be inferior to the control. All vaccines were well tolerated. No differences in rates of local and systemic reactions were seen between the investigational products and control. No serious adverse event was found to be related to the investigational vaccines.

Conclusion: Investigational vaccines are shown to be equally immunogenic and safe as the control vaccine.

Keywords: Hepatitis B, Hepatitis B virus, Immunogenicity, Safety, Vaccine

Introduction

Viral hepatitis is an international public health challenge comparable to other major infectious diseases, including human immunodeficiency virus (HIV), tuberculosis, and malaria [1]. Hepatitis B virus (HBV) immunization is a critical intervention for the elimination of HBV epidemics. More comprehensive provision of the existing, safe and effective HBV vaccine, including universal childhood vaccination and birth-dose delivery, will drastically reduce new hepatitis B infections, reducing chronic illness and death rates [1]. The strategy calls for an increase in routine childhood HBV vaccination coverage from 82% in 2015 to 90% by 2020, which will require strengthening overall childhood immunization programs and specific efforts to target HBV vaccination for those at increased risk [1].

Disease caused by HBV has a worldwide distribution. The endemicity of active HBV infection is reflected in the serologic prevalence of the hepatitis B surface antigen (HBsAg) in the general population of a defined geographical area. HBsAg prevalence of ≥8% defines highly endemic areas, prevalence of 5%–7% defines high intermediate, 2%–4% low intermediate, and <2% defines low endemic areas [2].

High(est) endemicity of hepatitis B (currently defined as ≥8% of the population HBsAg-positive) is found in areas of sub-Saharan Africa, South-East Asia, the Eastern Mediterranean countries, South and Western Pacific islands, the interior of the Amazon Basin, and in certain parts of the Caribbean; in these areas, up to 20% of the population may be chronically infected. Intermediate endemicity (2%–8% of the population HBsAg-positive) is located in South-Central and South-West Asia, Eastern and Southern Europe, the Russian Federation, and most of Central and South America [3].

Indonesia is a country with high endemicity of hepatitis B, the second largest in Southeast Asia country after Myanmar. Based on the results of study and blood screening of Palang Merah Indonesia (blood bank) donors, it is estimated that among 100 Indonesians, 10 of them have been infected with hepatitis B and C. So now there are an estimated 28 million Indonesians infected with hepatitis B [4].

Although other primates have been infected in laboratory conditions, HBV infection affects only humans. No animal or insect hosts or vectors are known to exist [5].

World Health Organization (WHO) has estimated that 84% of all infants worldwide received at least 3 doses of hepatitis B containing vaccine in 2015, and 39% of newborns received the birth dose. Vaccinating against hepatitis B has been associated with substantial reductions in acute and chronic hepatitis B infections and hepatocellular carcinoma mortality [6]. Strategic Advisory Group of Experts on Immunization recommends that all infants receive the birth dose during the first contact with health facilities at any time up to the time of the first primary dose. The birth dose given after 24 hours should be reported as a late birth dose vaccination [5]. The birth dose should be followed by 2 or 3 doses to complete the primary series [7].

Previous study of recombinant hepatitis B vaccine

Bio Farma Vaccine Institute had started producing the hepatitis B recombinant vaccine since the year 2000. The first study for hepatitis B vaccine recombinant in Indonesia was conducted by the Center for Disease Control, National Institute of Health Research and Development, and Ministry of Health. Two hundred and twenty infants were involved in this study and the recommendation from this study is to administer the hepatitis B vaccine in newborn infants [8]. Another study was conducted in 2001 in infants with different immunization schedules from Hasan Sadikin General Hospital in Bandung, West Java. This study's result was 95.4% of infants were protected in 0, 1, 2 schedule and 98.9% were protected in 0, 1, 6 schedule. Vaccination schedule 0, 1, 6 months of age-induced higher antibody compare to schedule 0, 1, 2 months of age [9]. A bridging study was conducted in 2005 to evaluate two different accelerated schedules 0, 7, 21 days (group I) and 0, 1, 2 months (group II), of hepatitis B vaccination in adults. There was no significant difference between those two groups in terms of seroprotection at day 365 (before booster vaccination) [10]. A Post-marketing Surveillance Study was conducted in healthy late adolescents not previously received hepatitis B vaccine. Subjects were administered three doses hepatitis B vaccine recombinant (20 μg of HBsAg). Anti HBs antibodies were detected in 95.5% of 113 subjects with anti HBs <10 mIU/mL, all vaccinations were well tolerated [11].

To meet the national immunization program's needs, it requires to maintain the capacity of monovalent hepatitis B production for national use and worldwide. Therefore, new hepatitis B bulk is needed to sustain the availability of this vaccine. This phase II (bridging) study was conducted to evaluate the immunogenicity and safety of new hepatitis B bulk compared to the registered product.

Materials and Methods

Study design and population

This trial was an experimental, randomized, double blind, prospective intervention study conducted by Faculty Medicine of Diponegoro University Semarang Indonesia. The study population are children, adolescents, and adults: 10–40 years old.

The subjects were grouped into three different batch of monovalent hepatitis B with new bulk and one group receive registered monovalent hepatitis B as as a control. The subjects were randomized per treatment group and was allocated by a randomization list so that to each subject, only one randomly assigned treatment group (A/B/C/D).

Subject recruited in primary school and junior high school at Tembalang Semarang and Nasional Diponegoro Hospital Semarang during September 2020 and finishing in June 2020.

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Inclusion criteria were healthy individuals as determined by clinical judgment, including medical history and physical examination; subjects/parents/guardian(s) have been informed regarding the study and signed the informed consent form/informed assent form; subject/parents/guardian(s) will commit to comply with the investigator's instructions and the trial schedule.

Subject concomitantly enrolled or scheduled to be enrolled in another trial; known history of hepatitis B contained vaccination in the last 10 years; evolving severe illness and/or chronic disease and fever (axillary temperature >37.5°C) within the 48 hours preceding enrollment; known history of allergy to any component of the vaccines (based on anamnesis); HBsAg positive; known history of immunodeficiency disorder (HIV infection, leukemia, lymphoma, or malignancy); with history of uncontrolled coagulopathy or blood disorders contraindicating intramuscular injection; subject who has received a treatment in the previous 4 weeks is likely to alter the immune response (intravenous immunoglobulins, blood-derived products, or corticosteroid therapy and other immunosuppressant) were excluded from the trial. Subjects with pregnancy and have been immunized with any vaccine within 4 weeks prior and expects to receive other vaccines within 4 weeks following immunization were also excluded.

Study procedure

Three days before the immunization visit, subjects were invited to receive information about the study procedure. After signing the informed consent form, and following the steps of evaluation, preimmunization blood was collected, and will come again for the immunization after receiving the result of HBsAg test. Each subject will receive 3 doses of vaccine with the interval of 1 month. The last visit will be 1 month after the last dose.

Study intervention

Each dose of recombinant hepatitis B vaccine (1 mL) is an inactivated HbsAg produced in yeast cells (*Hansenula polymorpha*) using recombinant DNA technology. It is a whitish liquid produced by culture genetically engineered yeast cell which carry the relevant gene of the HbsAg. The inactivated HbsAg (bulk) is imported from Serum Institute of India and then formulated and filled at Bio Farma batch 3660118S, 3660218S, and 3660318S. The control vaccine used in this trial was recombinant hepatitis B vaccine (1 mL), an inactivated HbsAg (bulk) imported from The Janssen Vaccine Corp. (Johnson & Johnson, New Brunswick, NJ, USA) and then formulated and filled at Bio Farma batch number 3660318. It had already been licensed. Both vaccines have similar packaging, so the trial could be done double-blind.

Safety and immunogenicity evaluation

The intensity, duration, and relation of each adverse event to the trial vaccines were evaluated at 30 minutes, 72 hours, and 28 days after injection by interviewing the subjects during the post-surveillance visits: V1, V2, V3, and V4. Any serious adverse events and the study process were reported and evaluated by the Data and Safety Monitoring Board (DSMB).

Four mL of blood were collected at visit V0 and V4. The V0 blood sample was divided into two aliquots. The first was used for the HbsAg test before recruitment, and the second aliquot will be stored as the pre-immunization samples. The V4 blood sample was stored as the post-immunization samples. Chemiluminescent Microparticle Immunoassay wase conducted in Commercial Laboratory in Bandung using kit reagent from Abbott Laboratories (Abbott Park, IL, USA). The anti-HBS was performed using Architect ausab reagent kit on architect i 1000sr (Abbott Laboratories). HBsAg was tested using same method but special kit for HBsAg detection.

Sample size and analysis

Sample size is determined based on 95% confidence interval (CI) and power of the test 80%. Using sample size formula for comparing two a population proportion. To evaluate immunogenicity and safety in three consecutive batches of recombinant hepatitis B vaccine with different source bulk, sample size is determined based on formula for studies one proportion. With the assumption that not all of the subject could complete the study, the total number of subjects will be added at least 20%, and will be involved 134 subjects for each group, and totally 536 subjects for the whole study, with expectation the sample size was suitable for both formulas.

The GMT and percentage of subjects with anti with anti HbsAg >10 IU/mL 28 days after last injection with their 95% CIs were described before and 1 month after the last dose. The randomization code was opened after the laboratory officially released the test results to the investigators. Vaccine safety was analyzed by computing the number and percentage of any adverse events experienced by subjects. All data were analyzed using IBM SPSS ver. 20.0 (IBM Corp., Armonk, NY, USA).

Reporting of serious adverse events

Every serious adverse event occurring throughout the trial reported to the DSMB, sponsor, and Ethics Committee, by the investigator as soon as he/she is alerted of it, i.e., within 24 hours, even if the investigator considers that the adverse event is not related to the treatment. Notification was made by phone message/email: then the investigator immediately sent the completed alert form to Ethics Committee. A copy of the alert form should be sent to sponsor.

Causality assessment

The causal relationship between the investigational product and each adverse event will be categorized by the investigator, sponsor, and DSMB with the WHO classification [12-14].

Ethics statement

The study obtained the ethical clearance from Diponegoro University Ethics Committee (no., 80/EC/FK UNDIP/III/2019), before starting the trial and approval of all amendments (no., 49/KEPK/FK UNDIP/XI/2019). Trial registration number is NCT 03919578 & INA-55NX6PA.

Results

All blinded samples at V0 and V4 have been tested from September 2019 to 1 March 2020. The randomization code was unblinded on March 4, 2020, which was attended by sponsor, investigator, and unblinded team. Vaccine code A was batch number 0218S; vaccine code B was batch number 0318 (control); vaccine code C was no batch 0118S; vaccine code D was

Table 1. Demographic characteristics

Characteristic	Group I				Group II			
	0118S	0218S	0318S	Control	0118S	0218S	0318S	Control
Total	67	67	67	67	67	67	67	67
Sex								
Female	41	38	43	52	38	47	38	45
Male	26	29	24	15	29	20	29	22
Mean age: adult (yr)/child (yr)/infant (mo)	14.34	14.04	14.12	14.15	26.88	26.28	26.13	27.13

Table 2. Number and percentage of subjects with anti-HbsAg ≥10 mIU/mL in children adolescent (group I)

Description	0118S (n=66)		0218S (n=67)		0318S (n=64)		Control (n=65)		p-value[a]
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
Anti-HbsAg ≥10 mIU/mL									0.502 (pre)
									1.000 (post)
No. (%SP)	16 (24.24)	166 (100.00)	15 (22.39)	66 (98.51)	9 (14.06)	64 (100.00)	13 (20.00)	65 (100.00)	
(95% CI)	(0.146–0.364)	(0.946–1.000)	(0.131–0.342)	(0.920–1.000)	(0.066–0.250)	(0.944–1.000)	(0.111–0.318)	(0.946–1.000)	

Values are presented as number of available observations (%), unless otherwise stated.
HBsAg, hepatitis B surface antigen; %SP, seroprotection rate; CI, confidence interval.
[a]p-value based on chi-square test/Fisher exact.

Table 3. Number and percentage of subjects with anti HbsAg ≥10 IU/mL in adults (group II)

Description	0118S (n=65)		0218S (n=66)		0318S (n=63)		Control (n=65)		p-value[a]
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
Anti-HbsAg ≥10 mIU/mL									0.502 (pre)
									1.000 (post)
No. (%SP)	16 (24.61)	65 (100.00)	19 (28.79)	66 (100.00)	26 (41.27)	63 (100.00)	24 (36.92)	64 (98.46)	
(95% CI)	(0.148–0.369)	(0.945–1.000)	(0.145–0.364)	(0.946–1.000)	(0.276–0528)	(0.943–1.000)	(0.226–0.466)	(0.917–1.000)	

Values are presented as number of available observations (%), unless otherwise stated.
HBsAg, hepatitis B surface antigen; %SP, seroprotection rate; CI, confidence interval.
[a]p-value based on chi-square test/Fisher exact.

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Table 4. Geometric mean of anti-HBsAg (GMT), percentage of subjects with increasing antibody titer ≥4 times and percentage of subjects with the transition of seronegative to seropositive

Description	0118S (n=131)		0218S (n=133)		0318S (n=127)		Control (n=130)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
GMT mIU/mL								
Mean	1.376	4,088.826	2.118	8,214.546	2.373	5,154.18	2.119	1,520.126
(95% CI)	(0.822–2.301)	(3,107.420–5,380.220)	(6,032.537–11,186.649)	(1,105.350–2,090.740)	(1.398–4.026)	(3,752.321–7,079.458)	(1.232–3.645)	(1,105.350–2,090.740)
Anti-HbsAg ≥10 mIU/mL (%SP)	32 (24.43)	131 (100.00)	34 (25.564)	132 (99.25)	35 (27.56)	127 (100.00)	37 (28.46)	129 (99.23)
Subjects with increasing antibody titer ≥4 times (%SC)	-	131 (100.00)	-	133 (100.00)	-	127 (100.00)	-	127 (97.69)
Subjects with transition of seronegative to seropositive (%SC)	-	99 (100.00)	-	98 (98.99)	-	92 (100.00)	-	92 (98.925)

Values are presented as mean (95% CI) or number of available observations (%), unless otherwise stated.
HBsAg, hepatitis B surface antigen; GMT, geometric mean titer; CI, confidence interval; %SP, seroprotection rate; %SC, seroconversion rate.

Table 5. Comparison of GMT, seroprotection, percentage of subjects with increasing antibody titer ≥4 times, and/or percentage of subjects with transition of seronegative to seropositive following primary series of investigational product compare to control

Description	Pre-vaccination					Post-vaccination				
	0118S (n=131)	0218S (n=133)	0318S (n=127)	0318 (n=130)	p-value	0118S (n=131)	0218S (n=133)	0318S (n=127)	0318 (n=130)	p-value
GMT (IU/mL)					0.41*					1.609e-13 (p<0.001)
Mean	1.376	2.119	2.373	2.119		4,088.826	8,214.546	5,154.18	1,520.126	
(95% CI)	(0.823–2.302)	(1.365–3.287)	(1.398–4.027)	(1.232–3.645)		(3,107.157–5,380.643)	(6,032.561–11,185.758)	(3,752.066–7,080.253)	(1,105.294–2,090.651)	
Anti-HbsAg ≥10 b					0.8782					0.8714
No.	32	34	35	37		131	132	127	129	
%SP	24.427	25.564	27.559	28.462		100	99.248	100	99.231	
p-value (compared to group B)	0.46	0.597	0.872			0.997	0.987	0.997	-	
Increasing antibody titer ≥4 times										0.029
No.	-	-	-	-	-	131	133	127	127	
%SC	-	-	-	-	-	100	100	100	97.69	
p-value (compared to group B)	-	-	-	-	-	0.748	0.913	0.799	-	
Transition of seronegative to seropositive										0.8452
No.	-	-	-	-	-	99	92	92	92	
%SC	-	-	-	-	-	100	98.99	100	98.925	

GMT, geometric mean titer; CI, confidence interval; HBsAg, hepatitis B surface antigen; %SP, seroprotection rate; %SC, seroconversion rate.

no batch 0318S.

As many as 536 subjects participated in this study. Fifteen participants were not completed the study because seven were terminated due to lost to follow-up, four terminated due to misallocation of vaccine code, and the other (n=4) withdrew because of moving away from the study area. Data on demographic and baseline characteristics are presented in Table 1. About the primary criteria regarding the number and percentage of subjects with anti-HBsAg ≥10 mIU/mL in children and adolescents (group I) are shown in Tables 2 and 3. Percentage of subjects with HbsAg ≥10 mIU/mL in children and adolescent (group I) 28 days after threes doses of vaccination was 100%, 98.51%, 100%, and 100% in batch number 0118S, 0218S, 0318S, and 0318 (control), respectively (Table 2).

Percentage of subjects with HbsAg ≥10 mIU/mL in adult group (group II) 28 days after threes doses of vaccination was 100%, 100%, 100%, and 98.46% in batch number 0118S, 0218S, 0318S, and control, respectively. After vaccination with hepatitis B vaccines recombinant, at least 98.51% children and adolescent, and 98.46% of adults reached protective level against hepatitis B. There was no significant different of seroprotection between group (Table 3). The following tables describe the immunogenicity of investigational products in all subjects (Table 4).

One month after three doses of recombinant hepatitis B vaccination, the GMT of anti-HBsAg in batch 0118S, 0218S, 0318S, and 0318 (control) was 4,088.826 IU/mL, 8,214.546 IU/mL, 5,154.18 IU/mL, and 1,520.126 IU/mL, respectively. Percentage of subjects with increasing antibody titer ≥4 times was 100%, 100%, 100%, and 97.69% in batch 0118S, 0218S, 0318S, and 0318, respectively. Percentage of subjects with transition of seronegative to seropositive was 100%, 98.99%, 100%, and 98.93% in batch 0118S, 0218S, 0318S, and 0318 (control), respectively. There was no significant difference in GMT ($p>0.05$), and transition of seronegative to seropositive ($p>0.05$) following Bio Farma's hepatitis B vaccine compare to control (Table 5).

One month after three doses of recombinant hepatitis B vaccination, the GMT of anti-HBsAg in batch 0118S, 0218S, and 0318S was 4,088.826 IU/mL, 8,214.546 IU/mL, and 5,154.18 IU/mL, respectively. Percentage of subjects with increasing antibody titer ≥4 times was 100%, 100%, and 100% in batch 0118S, 0218S, and 0318S, respectively. The percentage of subjects with the transition of seronegative to seropositive was 100%, 98.99%, and 100% in batch 0118S, 0218S, and 0318S, respectively. There was a significant difference in GMT ($p<0.05$)

Table 6. Comparison of GMT, seroprotection, percentage of subjects with increasing antibody titer ≥4 times, and/or percentage of subjects with transition of seronegative to seropositive following primary series between each number of investigational products

Description	Pre-vaccination				Post-vaccination			
	0118S (n=131)	0218S (n=133)	0318S (n=127)	p-value (between batches)	0118S (n=131)	0218S (n=133)	0318S (n=127)	p-value (between batches)
GMT (IU/mL)				0.2562				0.00296 ($p<0.05$)
Mean	1.376	2.118	2.373		4,088.826	8,214.546	5,154.18	1
(95% CI)	(0.823–2.302)	(1.365–3.287)	(1.398–4.027)		(3,107.157–5,380.643)	(6,032.561–11,185.758)	(3,752.066–7,080.253)	
Anti-HbsAg ≥10				0.845				
No.	32	34	35		131	132	127	
% SP	24.427	25.564	27.559		100	99.248	100	
Increasing antibody titer ≥4 times								1
No.	-	-	-		133	121	127	
% SC	-	-	-		100	100	100	
The transition of seronegative to seropositive								0.846
No.	-	-	-		99	98	92	
% SC	-	-	-		100	98.99	100	

GMT, geometric mean titer; CI, confidence interval; HBsAg, hepatitis B surface antigen; %SP, seroprotection rate; %SC, seroconversion rate.

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Table 7. Safety criteria according to age group

Description	Group I								p-value	Group II								p-value
	0118S		0218S		0318S		0318			0118S		0218S		0318S		0318		
	nEv	nSj (%)	nEv	nSj (%)	nEv	nSj (%)	nEv	nSj (%)		nEv	nSj (%)	nEv	nSj (%)	nEv	nSj (%)	nEv	nSj (%)	
After 1st immunization	n=67		n=67		n=67		n=67			n=67		n=67		n=67		n=67		
Any immediate reaction																		
Any immediate local reaction	22	18 (27.27)	26	20 (29.85)	12	10 (15.38)	26	22 (33.85)	0.120	45	29 (43.28)	34	23 (34.33)	19	17 (26.54)	26	22 (32.84)	0.250
Any immediate systemic event	5	4 (6.06)	4	2 (2.98)	2	1 (1.54)	2	1 (1.54)	0.471	8	6 (8.95)	9	8 (11.94)	4	4 (6.25)	7	7 (10.45)	0.741
Any delayed adverse event (31 min–72 hr)																		
Any delayed local reaction	11	11 (16.67)	14	12 (17.91)	15	12 (18.46)	14	9 (13.85)	0.876	3	3 (4.48)	9	8 (11.94)	6	5 (7.81)	9	6 (8.96)	0.469
Any delayed systemic event	3	3 (4.54)	7	5 (7.46)	12	8 (12.31)	5	5 (7.69)	0.420	4	3 (4.48)	6	5 (7.46)	8	7 (10.94)	15	12 (17.91)	0.059
Any delayed adverse event (4–28 day)																		
Any delayed local reaction	0	0	0	0	0	0	0	0	1.000	0	0	0	0	0	0	0	0	1.000
Any delayed systemic event	11	9 (13.64)	8	7 (10.45)	8	4 (6.15)	7	7 (10.77)	0.598	5	5 (7.46)	10	10 (14.92)	5	5 (7.81)	15	12 (17.91)	0.169
After 2nd immunization	n=66		n=67		n=64		n=66			n=67		n=67		n=65		n=66		
Any immediate reaction																		
Any immediate local reaction	21	18 (27.27)	27	24 (35.82)	22	19 (29.23)	27	24 (36.92)	0.615	14	14 (20.89)	20	18 (27.27)	19	17 (26.98)	25	22 (33.33)	0.466
Any immediate systemic event	5	4 (6.06)	8	8 (11.94)	11	10 (15.38	6	6 (9.23)	0.344	2	2 (2.98)	8	4 (6.06)	6	5 (7.94)	7	5 (7.58)	0.592

nEv, number of event; nSj, number of subject.

28 days following of investigational product between batch numbers of Bio Farma hepatitis B vaccine. There was no significant difference in seroprotection (p=1) and seroconversion (p>0.05) following of investigational product between batch number of Bio Farma Hepatitis B vaccinee (Table 6).

Safety

The following table shows the results of the evaluation regarding safety after dosing vaccination (Table 7). If the vaccine group was analysis between batch vaccines, most of adverse reactions were not significant difference (Table 8).

During the study, seven subjects were hospitalized, four for vaccine group and three from control groups. All events were reported to the Ethics Committee and DSMB. The causality assessment is coincidental and not related to vaccine or vaccination.

Discussion

In Indonesia, the hepatitis B vaccination program for infants has been implemented nationally since 1997 [15]. The plasma-derived vaccine was previously used until 1977, which was then replaced by a recombinant hepatitis B vaccine [16]. Several studies have shown differences in the protection between plasma-derived and recombinant vaccines, where recombinant vaccines have a higher protective effect [17]. The administration of the hepatitis B vaccine was carried out with the recommendation of one hepatitis B-0 (within the first 24 hours after birth) and three doses pentavalent vaccine (DPT [diphtheria-pertussis-tetanus]/hepatitis B/Haemophilus influenzae type b). In 2013, the coverage of hepatitis B-0 and three doses of DPT-hepatitis B in children aged 12–23 months were 79.1% and 75.6%, respectively. These numbers indicate there are still children who have not received the hepatitis B vaccine yet [18].

From our study, administering three doses of recombinant hepatitis B vaccine provided protection for 519 subjects (99.61%). This result is also supported by several other studies. Arjmand et al. [19] reported that administration of three doses of hepatitis B vaccine in a pentavalent vaccine resulted in protection against 98.3% (393 children) of the total subjects (400 children). Vaccine administration routes could also affect immunogenicity. Research in Japan on subjects aged 20–35 years old reported that the intramuscular route was more immunogenic than subcutaneous [20]. A study reported that the 4-dose hepatitis B vaccine produced higher protection than 3-doses

Table 8. Comparison of adverse events between investigational product and control

Description	0118S (n=131)		0218S (n=133)		0318S (n=127)		0318 (n=130)		p-value
	nEv	nSj (%)	nEv	nSj (%)	nEv	nSj (%)	nEv	nSj (%)	
After 1st immunization									
Any immediate reaction									
Any immediate local reaction	67	47 (35.34)	60	43 (32.09)	31	27 (20.93)	52	43 (33.33)	0.061
Any immediate systemic event	13	10 (7.52)	13	10 (7.46)	6	5 (3.88)	9	8 (6.06)	0.603
Any delayed adverse event (31 min–72 hr)									
Any delayed local reaction	14	14 (10.53)	23	20 (14.92)	21	17 (13.18)	23	15 (11.36)	0.699
Any delayed systemic event	7	6 (4.51)	13	10 (7.46)	20	15 (11.63)	20	17 (12.88)	0,069
Any delayed adverse event (4–28 day)									
Any delayed local reaction	0	0	0	0	0	0	0	0	1.000
Any delayed systemic event	16	14 (10.53)	18	17 (12.69)	13	9 (6.98)	22	19 (14.39)	0.280
After 2nd immunization									
Any immediate reaction									
Any immediate local reaction	35	32 (24.06)	47	42 (31.58)	41	36 (28.12)	52	46 (35.11)	0.258
Any immediate systemic event	7	6 (4.51)	16	12 (9.02)	17	15 (11.72)	13	11 (8,2)	0.208
Any delayed adverse event (31 min–72 hr)									
Any delayed local reaction	13	13 (9.77)	36	26 (19.55)	28	12 (9.37)	31	19 (14.5)	0.053
Any delayed systemic event	17	12 (9.02)	18	12 (9.02)	12	12 (9.37)	21	13 (9.92)	0.993
Any delayed adverse event (4–28 day)									
Any delayed local reaction	0	0	1	1 (0.75)	2	2 (1.56)	3	1 (1.76)	0.431
Any delayed systemic event	15	13 (9.77)	24	18 (13.53)	35	24 (18.75)	41	32 (24.43)	0.009
After 3rd immunization									
Any immediate reaction									
Any immediate local reaction	33	30 (22.9)	43	33 (24.81)	34	32 (25.2)	48	38 (29.23)	0.693
Any immediate systemic event	6	5 (3.82)	1	1 (0.75)	6	5 (3.94)	2	2 (1.54)	0.249
Any delayed adverse event (31 min–72 hr)									
Any delayed local reaction	29	19 (14.5)	30	23 (17.29)	28	16 (12.6)	36	18 (13.85)	0.748
Any delayed systemic event	6	5 (3.82)	10	8 (6.01)	12	11 (8.66)	16	11 (8.46)	0.353
Any delayed adverse event (4–28 day)									
Any delayed local reaction	1	1 (0.76)	0	0	2	2 (1.57)	1	1 (0.77)	0.386
Any delayed systemic event	25	17 (12.98)	18	16 (12.03)	18	17 (13.39)	28	21 (16.15)	0.796

nEv, number of event; nSj, number of subject.

administration [21]. Research in Singapore reported 3-dose hepatitis B vaccine, both monovalent and the vaccine combination showed promising results. There were no significant differences in reactivity between the four vaccine codes given in this study in both groups.

After administering 3 doses of the vaccine, two subjects (0.38%) did not undergo seroconversion, one subject from group I who received the vaccine code A and one subject from group II who received the vaccine code B. This number is still lower compared to several studies in the United States that reported up to 5%–10% of vaccine recipients did not respond [22]. A study in Iran, with a total of 538 subjects, reported there were 15.6% who failed to produce antibody titers >10 mIU/mL. This can be caused by several factors such as the environment and genetics, human leukocyte antigen (HLA), and immune tolerance of each individual [23]. A study in Iran, with a total of 538 subjects, reported there were 15.6% who failed to produce antibody titers >10 mIU/mL. This can be caused by several factors such as the environment and genetics, HLA, and immune tolerance of each individual [22].

In our study, the most common solicited local reaction was local pain. The majority of local pain was reported to occur within 30 minutes after any dose, with the group I was 15.6%–35.8% and group II was 21.5%–38.8%. These results are consistent with previous research conducted in India that found injection site pain of 11.5% of the total subjects as the most

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local reaction [24]. Other study in Thailand also reported that the most common adverse event was pain at the injection site (42.4%) [21]. The majority of local pain in our study was reported to occur within 30 minutes after any dose. This result is different from the study in 2014, which reported that local pain was found more in 1-day post-vaccination than 30 minutes after [25].

There was no significant difference between solicited systemic reactions. Most of the systemic reactions were below 10% after any dose. On the contrary, other study reported that most of the adverse events following immunization from 1,013 reports were general systemic reactions (47%) followed by local reactions (26%) [26]. Fatigue was the most common systemic reaction in both groups after the first and second doses. This finding is consistent with studies conducted in Malaysia and Thailand that reported fatigue as the most common systemic reaction following hepatitis B vaccination [21,27]. On the other hand, a study reported that fever was the main adverse event (58.9%) [10]. Induration and rash also noted as the main adverse events in Brazil [28].

Almost all of the local and systemic reactions were mild. Most of the reactions, local and systemic, resolved spontaneously in within 72 hours after vaccination. This also supported by a study conducted in Indonesia [29]. There were no significant differences of adverse events between the four vaccine codes.

Several serious adverse events reported during the study were considered unrelated to the study vaccine and the procedure. The investigational recombinant hepatitis B vaccine has proven high immunogenicity and an acceptable safety profile. This study supports the conclusion that the Bio Farma recombinant hepatitis B with new sources of hepatitis B bulk is a suitable complement for the licensed equivalent vaccines based on similar safety profiles and antibody responses to the vaccine antigens after 3-dose primary vaccination series.

In conclusion, all three investigational vaccines are shown to be equally immunogenic and safety as the control vaccine. There were no significant differences in immunogenicity results and adverse events between the investigational product and control. No significant difference of immunogenicity result and adverse events between each batch number of Bio Farma hepatitis B vaccine.

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