

Original Article

Enhancing immunogenicity: Evaluating the polio antigen immune response in hexavalent vaccine formulations

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Abstract

Hexavalent vaccines simplify childhood immunization by combining antigens against six diseases in a single formulation; however, the immunogenicity of each component must be evaluated. This study assessed poliovirus-specific immunoglobulin G (IgG) responses elicited by candidate hexavalent vaccines containing different doses of Sabin strain-derived inactivated poliovirus vaccine (sIPV). A preclinical study was conducted using 40 Wistar rats allocated to five groups: hexavalent vaccine containing low-, medium-, or high-dose sIPV; pentavalent vaccine combined with sIPV; or adjuvant control. Each group comprised four male and four female rats. The animals received primary immunization at week 0 and booster doses at weeks 4 and 8. Serotype-specific IgG titers were measured at weeks 0, 4, 8, and 12 using indirect enzyme-linked immunosorbent assay. Antibody responses were analyzed using repeated-measures analysis of variance. Titers against poliovirus serotypes 1, 2, and 3 were negligible at baseline and remained low at week 4. Marked responses were observed in all vaccinated groups at week 8, followed by lower titers at week 12. Significant between-group differences were detected at weeks 8 and 12 for serotype 1 ($p < 0.001$ and $p = 0.010$), serotype 2 (both $p < 0.001$), and serotype 3 (both $p = 0.010$). However, no consistent dose–response pattern was observed across serotypes, time points, or sexes. All animals survived and gained body weight during follow-up. The candidate hexavalent formulations elicited marked poliovirus-specific binding-IgG responses, supporting further preclinical evaluation using validated serological and serotype-specific neutralization assays.

Keywords: Poliovirus, hexavalent vaccine, humoral immunogenicity, immunoglobulin G, Sabin inactivated poliovirus vaccine

Introduction

Vaccination is among the most effective public health interventions for preventing infectious diseases and reducing childhood morbidity and mortality. Vaccines are biological preparations containing antigens derived from attenuated or inactivated pathogens, purified or recombinant microbial components, inactivated toxins (toxoids), or genetic material that induces antigen production. These antigens may be formulated with adjuvants and other excipients to enhance immune responses and maintain product stability [1]. However, the increasing complexity of childhood immunization schedules can create logistical and programmatic barriers, potentially



contributing to delayed or incomplete vaccination [2]. Combination vaccines address some of these challenges by incorporating multiple antigens into a single formulation, thereby reducing the number of injections and healthcare visits required while simplifying vaccine delivery.

Hexavalent vaccines provide protection against diphtheria, tetanus, pertussis, hepatitis B, *Haemophilus influenzae* type b (Hib), and poliomyelitis [3]. Their use may improve the timeliness and efficiency of routine childhood immunization, particularly in settings where missed opportunities for vaccination and delayed administration remain important concerns. In Indonesia, coverage of individual vaccine components remains variable, and delayed receipt of inactivated poliovirus vaccine (IPV) and diphtheria–tetanus–pertussis–hepatitis B–Hib vaccines has been reported [2,4]. A locally developed hexavalent vaccine could therefore support a more streamlined immunization schedule. Nevertheless, combining several antigens within a single product requires rigorous evaluation to ensure that the immunogenicity of each component is preserved [5,6].

The poliovirus component is particularly important because sustained population immunity is essential for preventing poliovirus transmission and supporting global eradication. IPV contains antigens from poliovirus serotypes 1, 2, and 3 and induces systemic humoral immunity without the risk of vaccine-associated paralytic poliomyelitis [7]. Sabin strain-derived IPV (sIPV) has emerged as an alternative to conventional wild-type strain-derived IPV because it can be manufactured under lower biosafety risks while retaining the capacity to induce immune responses against all three poliovirus serotypes [8]. Incorporating sIPV into a hexavalent formulation may consequently facilitate the development of an integrated vaccine suitable for routine immunization programs.

The immunogenic performance of an antigen administered alone cannot, however, be assumed to remain unchanged when it is incorporated into a multivalent formulation. Physicochemical interactions among antigens, adjuvants, stabilizers, and preservatives may affect antigen integrity, adsorption, accessibility, and immune recognition [9,10]. Immunological interference may also arise from competition among antigens for processing and presentation, whereas favorable interactions with other components or adjuvants may enhance the response. These considerations are especially relevant to sIPV because variations in antigen content and formulation could produce different antibody responses to the three poliovirus serotypes. Preclinical dose-comparison studies are therefore required to determine whether candidate hexavalent formulations elicit adequate and consistent polio-specific immune responses before further clinical development.

This study aimed to evaluate polio-specific immunoglobulin G responses elicited by candidate hexavalent vaccines containing low, medium, or high doses of sIPV in Wistar rats. Antibody responses against poliovirus serotypes 1, 2, and 3 were assessed longitudinally after primary immunization and two booster doses and compared with responses elicited by a pentavalent vaccine administered with sIPV and by an adjuvant control. The findings might provide preclinical evidence to inform dose selection and the continued development of an sIPV-containing hexavalent vaccine.

Methods

Study design and setting

A controlled preclinical study was conducted from December 2024 to May 2025 to evaluate polio-specific humoral immune responses elicited by candidate hexavalent vaccines containing different doses of sIPV. The experimental procedures were conducted at Bio Farma (Persero), Bandung, Indonesia. Serum specimens were analyzed at the Biosafety Level 3 Laboratory of the Professor Nidom Foundation, Surabaya, Indonesia. The study was performed in accordance with the applicable World Health Organization guidelines, including Technical Report Series No. 927, Annex 1, and Technical Report Series No. 987, Annex 2.

Experimental animals and husbandry

A total of 40 specific pathogen-free Wistar rats (*Rattus norvegicus*), comprising 20 males and 20 females aged 6–8 weeks, were used. Animals were eligible for inclusion when they were clinically

healthy, met the prespecified age and body-weight criteria, and exhibited no signs of disease or other conditions that could interfere with the experimental intervention or outcome assessment. The animals were maintained in an accredited Animal Biosafety Level 3 facility at a temperature of approximately 24°C and relative humidity of approximately 60%. Standard laboratory feed and drinking water were provided ad libitum throughout the study.

Experimental groups

A total of 40 Wistar rats, consisting of 20 males and 20 females, were allocated to five experimental groups, with each group comprising four males and four females. Group 1 received the candidate hexavalent vaccine containing low-dose sIPV; group 2 received the candidate hexavalent vaccine containing medium-dose sIPV; group 3 received the candidate hexavalent vaccine containing high-dose sIPV; group 4 received the pentavalent Bio Farma vaccine administered with sIPV as an active comparator; and group 5 received the adjuvant formulation without vaccine antigens as the control.

Vaccine formulations

The candidate hexavalent vaccine, comprising diphtheria, tetanus, whole-cell pertussis, hepatitis B, Hib, and sIPV antigens (DTwP–HepB–Hib–sIPV), was manufactured by Bio Farma, Bandung, Indonesia. Each dose contained diphtheria antigen at ≥ 60 IU, tetanus antigen at ≥ 40 IU, whole-cell pertussis antigen at ≥ 4 IU, hepatitis B surface antigen at ≥ 10 μ g, and Hib antigen at ≥ 10 μ g. These antigen quantities were maintained across the three candidate formulations; only the sIPV content differed.

The high-dose formulation contained 15, 45, and 45 D-antigen units (DU) of sIPV serotypes 1, 2, and 3, respectively. The medium-dose formulation contained 12, 38, and 38 DU of sIPV serotypes 1, 2, and 3, respectively. The low-dose formulation contained two-thirds of the corresponding sIPV content in the high-dose formulation. Each formulation contained 0.33 mg of aluminum per dose as an aluminum phosphate adjuvant. The vaccine formulations were stored at 2–8°C and handled in accordance with standard preclinical laboratory procedures before administration.

Vaccine preparation

Hepatitis B, tetanus, and diphtheria antigens were adsorbed onto the aluminum phosphate adjuvant under controlled conditions and continuously mixed to achieve a homogeneous suspension. Whole-cell pertussis, Hib, and sIPV antigens representing poliovirus types 1, 2, and 3 were subsequently added sequentially. Water for injection was added to obtain the target volume, followed by final mixing and pH adjustment. The final formulations had a specified pH range of 6.0–7.5.

Immunization schedule

Each animal received three intramuscular administrations: primary immunization on day 0, the first booster at week 4, and the second booster at week 8. Each administration had a total volume of 0.5 mL, divided equally between the two thigh muscles at 0.25 mL per injection site. Week 12 was designated as the final observation point.

Blood collection and serum preparation

Blood samples were collected before each vaccine administration at weeks 0, 4, and 8 through the jugular vein. The final blood sample was collected at week 12 by cardiac puncture after euthanasia. At the end of the study, the animals were euthanized by an intraperitoneal overdose of ketamine (75 mg/kg) and xylazine (10 mg/kg). Blood specimens were collected into plain tubes and allowed to clot. Serum was separated by centrifugation at 3,000 rpm for 15 min and retained for the measurement of polio-specific immunoglobulin G (IgG).

Measurement of polio-specific IgG

IgG antibodies against sIPV serotypes 1, 2, and 3 were measured separately using an indirect enzyme-linked immunosorbent assay (ELISA). Serotype-specific coating solutions were prepared in carbonate buffer. For sIPV type 1, 2.0 mL of antigen was combined with 8.0 mL of carbonate

buffer; for type 2, 0.8 mL of antigen was combined with 9.2 mL of carbonate buffer; and for type 3, 1.6 mL of antigen was combined with 8.4 mL of carbonate buffer. Each preparation produced a final coating volume of 10 mL. The wells of a 96-well microplate were coated with 100 μ L of the corresponding antigen solution and incubated overnight at 4°C. The plates were washed three times with phosphate-buffered saline containing 0.05% Tween 20. The wells were then blocked with 100 μ L blocking buffer (0.5% bovine serum albumin (BSA) in PBS) and incubated at 37°C for 1 h, followed by five washing steps.

Serum samples were heat-inactivated at 56°C for 30 min and diluted 1:100 in sample buffer containing 0.05% Tween 20, 0.5% bovine serum albumin, and 0.5 M sodium chloride in phosphate-buffered saline. Diluted serum specimens and positive controls were added at 100 μ L per well and incubated for 1 h at 37°C. After three washes, 100 μ L of horseradish peroxidase-conjugated goat anti-rat IgG diluted 1:1,000 was added to each well, followed by incubation for 1 h at 37°C. The plates were washed three times, and 75 μ L of tetramethylbenzidine substrate was added. Following incubation for 25–30 min at room temperature in the dark, the reaction was terminated using 75 μ L of 1 M stop solution. Absorbance was measured immediately at 450 nm using a BioRad iMark™ Microplate Absorbance Reader. Optical-density measurements were converted into antibody-titer units using the prespecified calculation method.

Outcomes

The primary outcome was the longitudinal polio-specific IgG response against sIPV serotypes 1, 2, and 3, measured at weeks 0, 4, 8, and 12. Secondary outcomes included changes in body weight, clinical observations, and survival during the follow-up period.

Statistical analysis

Poliovirus-specific IgG antibody titers were summarized as mean $\pm$ standard deviation (SD) according to experimental group, sex, serotype, and measurement time point. Changes in antibody titers across weeks 0, 4, 8, and 12 were evaluated separately for each experimental group and sex using repeated-measures analysis of variance (ANOVA). Differences among experimental groups were also assessed at each measurement time point. Body weight, clinical observations, and survival were summarized descriptively. All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant. Analyses were performed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA).

Results

IgG antibody responses against sIPV serotypes

Poliovirus-specific IgG responses against sIPV serotypes 1, 2, and 3 were evaluated at baseline (week 0) and at weeks 4, 8, and 12 across the five experimental groups (**Figure 1**). Baseline IgG titers were negligible in all groups. Following immunization, IgG titers increased substantially in all vaccinated groups and generally rose further after booster administration. Although formulations containing higher sIPV doses tended to elicit higher IgG responses, a consistent dose–response pattern was not observed across all dose groups, serotypes, time points, and sexes. Male and female rats exhibited broadly comparable temporal response patterns. By contrast, the adjuvant-control group showed only minimal IgG responses throughout the observation period, supporting the induction of polio-specific humoral responses by the sIPV-containing vaccine formulations (**Figure 1**).

Comparison of antibody responses against sIPV serotypes among experimental groups

For poliovirus serotype 1, antibody titers were negligible at baseline and remained low at week 4, without significant between-group differences ($p = 0.420$ and $p = 0.450$, respectively). Titers were substantially higher in vaccinated groups at week 8, resulting in a significant between-group difference ($p < 0.001$), which persisted at week 12 ($p = 0.010$) despite lower titers. Significant temporal differences were observed in all vaccinated groups (all $p < 0.001$), but not in male or female controls ($p = 0.190$ and $p = 0.090$, respectively) (**Table 1**).

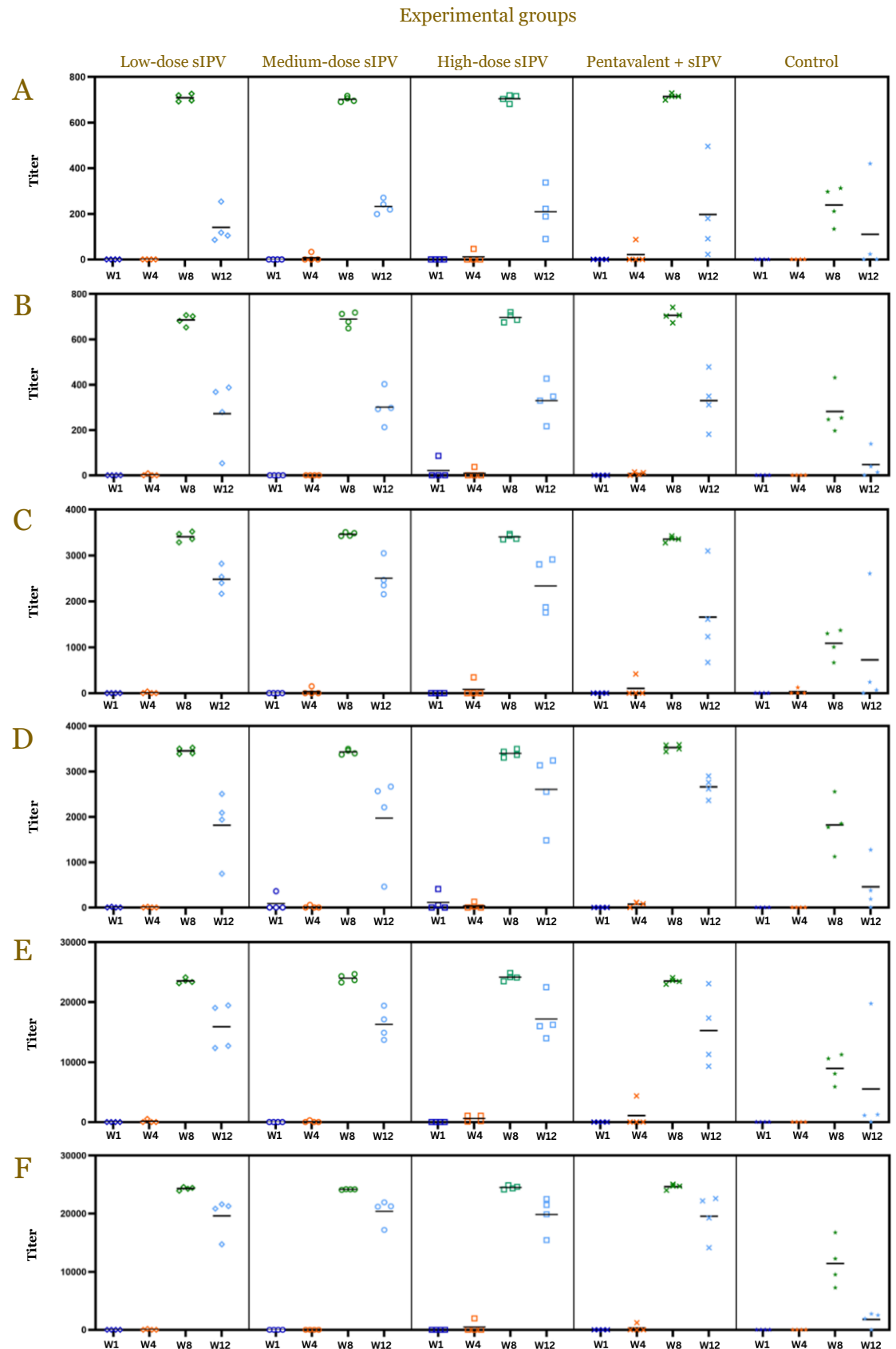


Figure 1. Poliovirus serotype-specific IgG antibody responses according to experimental group, sex, and measurement time point in Wistar rats. (A) IgG antibody titer of sIPV-1 in male rat. (B) IgG antibody titer of sIPV-1 in female rat. (C) IgG antibody titer of sIPV-2 in male rat. (D) IgG antibody titer of sIPV-2 in female rat. (E) IgG antibody titer of sIPV-3 in male rat. (F) IgG antibody titer of sIPV-3 in female rat. Data are presented as mean $\pm$ SD (n=4 animals per group).

Table 1. Poliovirus serotype 1-specific IgG antibody titers according to experimental group, sex, and measurement time point

Group	Sex	IgG antibody titers (mean ± standard deviation)				p-value ^a
		Week 0	Week 4	Week 8	Week 12	
Low	Male	0.00±0.00	0.00±0.00	70,882.50±1,680.97	14,069.75±7,657.90	<0.001**
	Female	0.00±0.00	230.50±461.00	68,585.00±2,440.71	27,241.00±15,355.65	<0.001**
Medium	Male	0.00±0.00	824.75±1,649.50	70,248.00±1,184.28	23,293.50±3,101.50	<0.001**
	Female	0.00±0.00	0.00±0.00	68,925.25±3,228.00	30,159.75±7,787.0	<0.001**
High	Male	0.00±0.00	1,165.50±2,331.00	70,495.00±1,721.50	20,955.75±10,189.28	<0.001**
	Female	2,150.25±4,300.50	938.50±1,877.00	69,666.50±2,027.63	33,051.75±8,657.13	<0.001**
Pentavalent + sIPV	Male	0.00±0.00	2,180.75±4,361.50	71,470.25±1,244.66	19,726.75±20,921.59	<0.001**
	Female	0.00±0.00	758.25±693.68	70,602.00±2,800.02	33,038.50±12,204.08	<0.001**
Placebo	Male	0.00±0.00	0.00±0.00	23,888.00±8,310.97	11,079.00±20,614.07	0.190
	Female	0.00±0.00	0.00±0.00	28,216.00±10,235.07	4,796.75±6,283.29	0.090
	p-value ^a	0.420	0.450	<0.001**	0.010*	

IgG: immunoglobulin G; sIPV: Sabin strain-derived inactivated poliovirus vaccine

^a Analyzed using analysis of variance (ANOVA)* Statistically significant at $p < 0.05$ ** Statistically significant at $p < 0.01$

Table 2. Poliovirus serotype 2-specific IgG antibody titers according to experimental group, sex, and measurement time point

Group	Sex	IgG antibody titers (mean ± standard deviation)				p-value ^a
		Week 0	Week 4	Week 8	Week 12	
Low	Male	0.00±0.00	975.50±1,951.00	340,800.75±10,564.62	247,953.50±27,402.76	<0.001**
	Female	382.25±764.50	382.25±764.50	345,217.50±7,034.33	181,934.75±75,454.69	<0.001**
Medium	Male	0.00±0.00	3,777.25±7,554.50	346,272.25±4,022.46	250,392.25±38,426.32	<0.001**
	Female	9,017.75±18,035.50	1,503.00±3,006.00	343,174.00±5,590.79	197,491.75±102,705.96	<0.001**
High	Male	0.00±0.00	8,622.25±17,244.50	340,372.50±5,773.20	233,583.00±60,539.74	<0.001**
	Female	11,509.50±19,772.41	3,797.00±6,220.85	339,976.75±8,274.57	260,313.00±80,480.71	<0.001**
Pentavalent + sIPV	Male	0.00±0.00	10,468.00±20,936.00	335,736.00±6,003.31	165,488.00±103,700.63	<0.001**
	Female	0.00±0.00	7,475.25±5,169.55	352,633.50±7,132.85	265,817.50±22,793.74	<0.001**
Placebo	Male	0.00±0.00	3,052.00±6,104.00	108,698.00±32,246.17	72,748.00±125,478.10	0.170
	Female	0.00±0.00	0.00±0.00	182,560.75±58,289.21	45,951.75±56,383.32	0.030*
	p-value ^a	0.490	0.320	<0.001**	<0.001**	

IgG: immunoglobulin G; sIPV: Sabin strain-derived inactivated poliovirus vaccine

^a Analyzed using analysis of variance (ANOVA)* Statistically significant at $p < 0.05$ ** Statistically significant at $p < 0.01$

Table 3. Poliovirus serotype 3-specific IgG antibody titers according to experimental group, sex, and measurement time point

Group	Sex	IgG antibody titers (mean ± standard deviation)				p-value ^a
		Week 0	Week 4	Week 8	Week 12	
Low	Male	0.00±0.00	13,690.25±27,380.50	2,356,065.25±42,706.07	1,589,543.50±388,893.47	<0.001**
	Female	0.00±0.00	4,559.75±9,119.50	2,431,593.25±28,245.97	1,963,022.00±327,412.82	<0.001**
Medium	Male	0.00±0.00	7,603.25±15,206.50	2,400,413.00±62,427.26	1,628,673.75±252,856.13	<0.001**
	Female	0.00±0.00	0.00±0.00	2,417,555.50±5,845.19	2,040,195.75±216,047.83	<0.001**
High	Male	0.00±0.00	59,543.50±54,838.03	2,415,630.50±56,615.35	1,716,934.75±369,192.64	<0.001**
	Female	0.00±0.00	49,559.75±99,119.50	2,450,630.75±31,451.98	1,984,543.25±312,735.06	<0.001**
Pentavalent + sIPV	Male	0.00±0.00	119,593.00±212,191.40	1,833,994.50±1,004,463.15	1,170,636.75±835,661.31	0.020*
	Female	0.00±0.00	31,299.00±62,598.00	2,464,761.00±44,492.75	1,956,065.25±388,882.28	<0.001**
Placebo	Male	0.00±0.00	0.00±0.00	896,282.75±245,719.75	552,538.00±951,062.54	0.140
	Female	0.00±0.00	0.00±0.00	1,145,848.00±407,324.34	179,929.50±124,914.95	0.010*
	p-value ^a	1.000	0.060	0.010*	0.010*	

IgG: immunoglobulin G; sIPV: Sabin strain-derived inactivated poliovirus vaccine

^a Analyzed using analysis of variance (ANOVA)* Statistically significant at $p < 0.05$ ** Statistically significant at $p < 0.01$

For poliovirus serotype 2, antibody titers were negligible at baseline and remained low at week 4, without significant between-group differences ($p=0.490$ and $p=0.320$, respectively) (Table 2). Titers were substantially higher in vaccinated groups at week 8, resulting in significant between-group differences at weeks 8 and 12 (both $p<0.001$). Significant temporal differences were observed in all vaccinated groups (all $p<0.001$). Among controls, the temporal difference was significant in female rats ($p=0.030$), but not in male rats ($p=0.170$) (Table 2).

For poliovirus serotype 3, antibody titers were negligible at baseline and remained relatively low at week 4, without significant between-group differences ($p=1.000$ and $p=0.060$, respectively) (Table 3). Titers were substantially higher in vaccinated groups at week 8, with significant between-group differences observed at weeks 8 and 12 (both $p=0.010$). Significant temporal differences were observed in all vaccinated groups (all $p\leq 0.020$). Among controls, the temporal difference was significant in female rats ($p=0.010$), but not in male rats ($p=0.140$) (Table 3).

Body weight

From week 1 to week 12, body weight increased consistently in both male and female rats across all groups (Groups 1–5), with no observed decrease. This finding indicates that administration of the hexavalent vaccine containing the polio antigen did not adversely affect body weight in either male or female rats (Figure 2).

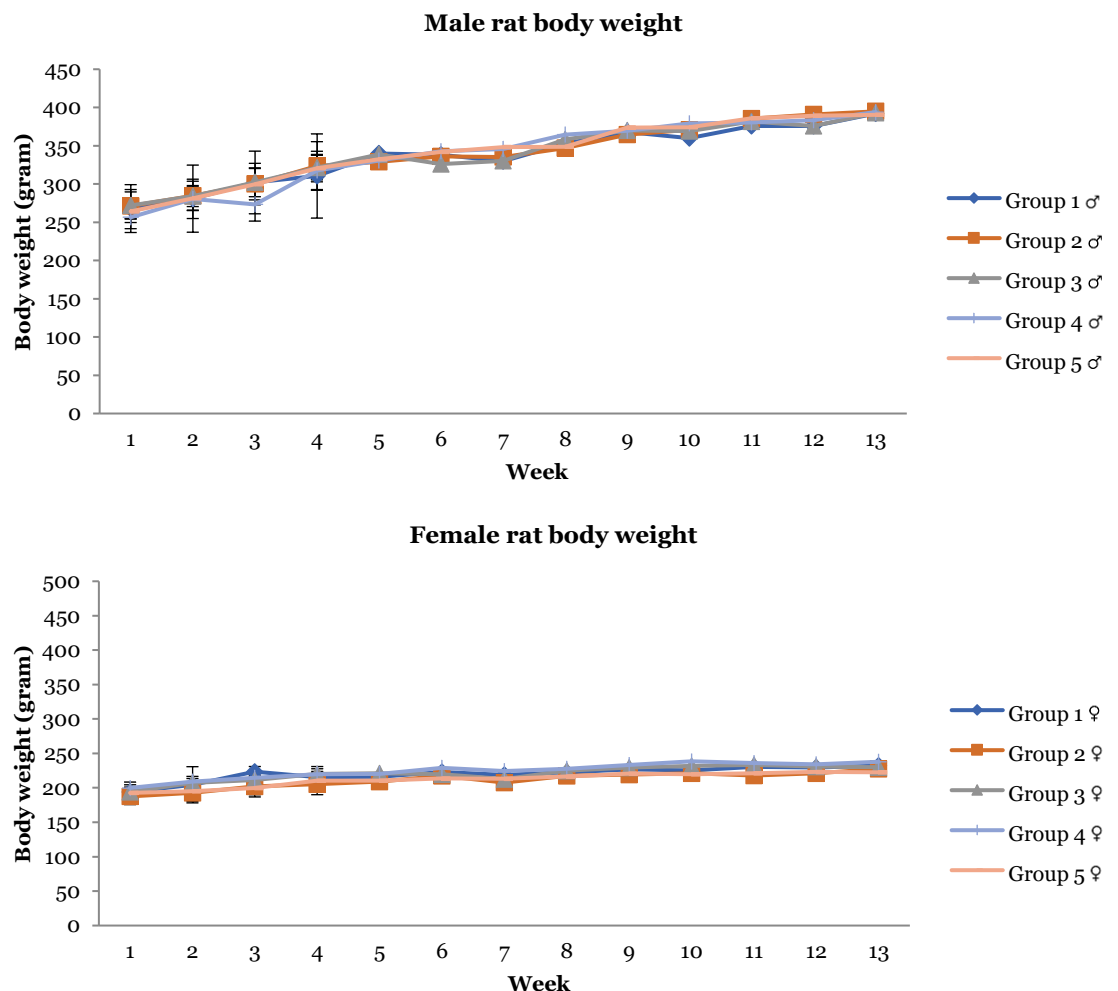


Figure 2. Temporal changes in body weight according to experimental group and sex during the 12-week observation period in Wistar rats. Body weight was measured weekly from weeks 1 to 12. Data are presented as mean $\pm$ standard deviation (SD; $n=4$ per sex in each group). Group 1: low-dose sIPV hexavalent vaccine; group 2: medium-dose sIPV hexavalent vaccine; group 3: high-dose sIPV hexavalent vaccine; group 4: pentavalent Bio Farma vaccine combined with sIPV; and group 5: adjuvant control. sIPV: Sabin strain-derived inactivated poliovirus vaccine.

Daily monitoring

Daily monitoring from week 1 to week 12 showed that all treatment groups remained in normal condition following administration of the hexavalent vaccine containing the polio antigen. The mucosa of both male and female rats appeared pink, indicating normal mucosal status. Bright yellow urine with a pH ranging from 6.5 to 7 and regular daily urination suggested normal kidney function [11]. No hair loss was observed. The fecal consistency was neither too soft nor too hard, indicating normal digestive function. All rats survived throughout the observation period from week 1 to week 12.

Discussion

This study evaluated poliovirus-specific IgG responses elicited by candidate hexavalent vaccines containing different doses of sIPV. All vaccinated groups demonstrated marked antibody responses against poliovirus serotypes 1, 2, and 3, with the highest mean titers generally observed at week 8, four weeks after the second booster dose. Antibody titers were lower at week 12 but remained higher than the corresponding baseline values. These findings indicate that the sIPV-containing formulations elicited measurable short-term humoral responses in Wistar rats. However, a consistent dose-response pattern was not observed across the three serotypes, measurement time points, and sexes.

IgG is the predominant antibody isotype generated following vaccination and is commonly used to evaluate vaccine-induced humoral immunogenicity [12,13]. Following antigen exposure, activated B lymphocytes differentiate into antibody-secreting plasma cells, whereas a subset develops into memory B cells that contribute to responses upon subsequent antigen exposure [14]. The marked antibody responses observed at week 8 are therefore compatible with secondary humoral immune activation following booster administration. Nevertheless, memory B-cell formation was not directly evaluated; thus, the observed antibody kinetics should not be interpreted as evidence of long-term immunological memory. The antibody responses differed considerably among the three poliovirus serotypes, with the highest absolute titers observed for serotype 3, followed by serotypes 2 and 1. However, direct comparisons of absolute titers across serotypes should be interpreted cautiously because the serotype-specific coating-antigen concentrations differed. Indirect ELISA measurements may also be influenced by antigen coating, serum dilution, nonspecific binding, background signals, and the method used to convert optical-density measurements into antibody titers [15]. Therefore, the apparent differences among serotypes may reflect both biological variation and assay-related factors.

Considerable antibody measurements were also recorded in the adjuvant-control group, particularly for serotypes 2 and 3 at weeks 8 and 12. Because the animals were maintained under specific pathogen-free conditions and the control formulation did not contain poliovirus antigens, environmental exposure to poliovirus is unlikely. The observed measurements may instead reflect nonspecific binding, cross-reactivity, background ELISA reactivity, inter-assay variability, or limitations in the antibody-titer calculation. Adjuvants can stimulate innate immune responses, but such stimulation would not be expected to generate genuinely poliovirus-specific antibodies in the absence of the corresponding antigen [16]. These findings warrant verification using serial serum dilutions, predefined assay cutoffs, blank-corrected optical densities, appropriate negative controls, and confirmatory functional assays.

Significant differences among experimental groups were observed at weeks 8 and 12 for all three serotypes. Nevertheless, post hoc pairwise comparisons were not reported; therefore, it cannot be concluded that a particular formulation produced significantly higher titers than another. Although higher-dose formulations occasionally produced higher mean titers, no consistent dose-dependent pattern was evident. The findings may indicate that the lower antigen dose was sufficient to approach the maximum response measurable by the assay or that the assay reached its upper analytical range. Alternatively, interactions among antigens, adjuvants, and other formulation components may have influenced antigen availability and immune recognition. Combination vaccines consequently require careful evaluation to ensure the safety and immunogenicity of each component within the final formulation [17].

Aluminum phosphate was used as the adjuvant in the candidate formulations. Aluminum-containing adjuvants can enhance antigen uptake, antigen presentation, and antibody

production; however, their effects depend on antigen adsorption, formulation characteristics, and antigen–adjuvant compatibility [18]. In a multivalent formulation, differences in antigen properties and adsorption may affect the accessibility and processing of individual antigens. However, antigen competition or synergy was not directly investigated in this study because responses to the other vaccine components were not measured and sIPV administered alone was not included as a comparator. Therefore, the present findings cannot establish either immunological interference or synergy between sIPV and the diphtheria, tetanus, pertussis, hepatitis B, and Hib components.

The temporal antibody pattern may be biologically compatible with germinal-center reactions, affinity maturation, and immunoglobulin class switching after repeated immunization [19]. Memory B cells may also contribute to more rapid and pronounced responses after subsequent antigen exposure [20]. Nevertheless, these mechanisms were not directly assessed. The study did not measure antibody avidity, IgG subclasses, memory B cells, cytokine profiles, or cellular immune responses. Accordingly, the antibody kinetics should be interpreted as changes in total antigen-binding IgG rather than evidence of affinity maturation, T-helper-cell polarization, or enhanced immune-memory formation.

Antibody titers were generally lower at week 12 than at week 8, suggesting contraction of the circulating antibody response after its apparent peak. However, follow-up ended at week 12; therefore, the persistence of the antibody response could not be determined. Moreover, the indirect ELISA measured antigen-binding IgG rather than poliovirus-neutralizing antibodies. Binding-antibody concentrations do not necessarily indicate functional neutralization or protection. Serotype-specific neutralization assays are therefore required to determine whether the elicited antibodies can prevent poliovirus infection.

Body weight increased throughout the observation period, all animals survived, and no overt clinical abnormalities were reported. These observations provide preliminary evidence that the formulations were tolerated under the experimental conditions. However, safety outcomes were evaluated descriptively and were not supported by hematological, biochemical, histopathological, or injection-site assessments. Normal weight gain and survival should therefore not be interpreted as establishing a comprehensive preclinical safety profile.

These findings provide preliminary evidence supporting further preclinical development of an sIPV-containing hexavalent vaccine. Nevertheless, regulatory evaluation of combination vaccines requires validated assays demonstrating that each antigen retains adequate potency and immunogenicity within the final formulation. Lot-to-lot consistency studies are also necessary to ensure that manufacturing variability does not affect antigen content, adsorption, stability, or biological activity [21]. Animal immunogenicity data may support vaccine development, but their relevance to protection depends on the use of validated immune correlates and functional assays [22].

The novelty of this study lies in the longitudinal evaluation of poliovirus serotype-specific IgG responses elicited by candidate Bio Farma hexavalent vaccine formulations containing different doses of sIPV. Although antigen competition, affinity maturation, memory B-cell responses, and T-helper-cell polarization may contribute to the observed antibody kinetics, these mechanisms were not directly assessed. Therefore, the findings primarily provide preliminary preclinical evidence of binding-antibody responses to support dose selection and further development of the candidate multivalent vaccine.

Conclusion

The candidate hexavalent vaccine formulations containing sIPV elicited marked binding-IgG responses against poliovirus serotypes 1, 2, and 3 in Wistar rats, with antibody titers generally reaching their highest levels at week 8. However, no consistent dose–response pattern was observed across formulations, serotypes, time points, or sexes. Further studies using validated serological methods and serotype-specific neutralization assays are required to determine the functional and protective capacity of the induced antibody responses.

Ethics approval

The study protocol was reviewed and approved by the Animal Care and Use Committee of Institut Teknologi Bandung, Bandung, Indonesia (approval number: KEP/I/2024/VIII/H1507241K/IVHB). All experimental procedures were conducted in accordance with institutional animal-welfare requirements and applicable guidelines for the care and use of laboratory animals.

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Competing interests

The authors declare no conflicts of interest.

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Underlying data

The data presented in this study are available on reasonable request from the corresponding author.

Declaration of artificial intelligence use

AI-based language model, ChatGPT, was used solely for language refinement, including improving grammar, sentence structure, and readability of the manuscript. The authors confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

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