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DETECTION OF PRIMARY ANTIBODIES AGAINST SUPERNATANT ANTIGENS IN PIGS VACCINATED WITH INACTIVATED *STREPTOCOCCUS SUIS* USING DIFFERENT ADJUVANTS

Deteksi Antibodi Primer Terhadap Antigen Supernatan Pada Babi yang Divaksinasi *Streptococcus suis* Inaktif Dengan Adjuvan Berbeda

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Abstract

Streptococcus suis is a pathogenic bacterium that causes disease in pigs and has zoonotic potential, leading to economic losses in the livestock industry and posing risks to human health. This experimental study employed a factorial completely randomized design to evaluate the primary antibody response to supernatant antigens in pigs vaccinated with inactivated *Streptococcus suis* vaccines formulated with different adjuvants. Twelve pigs were randomly assigned to three treatment groups: a control group (receiving only adjuvant without antigen), a vaccine group with Montanide ISA 201 VG adjuvant, and a vaccine group with Montanide Gel 01 adjuvant. Serum samples were collected weekly for five weeks, and antibody titers were measured using an enzyme-linked immunosorbent assay (ELISA).The results demonstrated that both adjuvanted vaccines significantly increased antibody titers compared to the control group. Antibody production was detectable from the second week post-vaccination, reaching peak levels in the fourth week. Comparative analysis revealed that Montanide ISA 201 VG induced slightly higher antibody responses than Montanide Gel 01. These findings support the use of inactivated *Streptococcus suis* vaccines with adjuvants as an effective preventive strategy to enhance immunity in swine populations and reduce the risk of zoonotic transmission.

Keywords: *Streptococcus suis*; inactivated vaccine; adjuvant; ELISA; antibody titer.

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Abstrak

Streptococcus suis adalah bakteri patogen yang menyebabkan penyakit pada babi dan memiliki potensi zoonosis, yang dapat menyebabkan kerugian ekonomi dalam industri peternakan serta menimbulkan risiko terhadap kesehatan manusia. Studi eksperimental ini menggunakan rancangan acak lengkap faktorial untuk mengevaluasi respons antibodi primer terhadap antigen supernatan pada babi yang divaksinasi dengan vaksin *Streptococcus suis* inaktif yang diformulasikan dengan berbagai adjuvan. Dua belas ekor babi dibagi secara acak ke dalam tiga kelompok perlakuan: kelompok kontrol (hanya menerima adjuvan tanpa antigen), kelompok vaksin dengan adjuvan Montanide ISA 201 VG, dan kelompok vaksin dengan adjuvan Montanide Gel 01. Sampel serum dikumpulkan setiap minggu selama lima minggu, dan kadar antibodi diukur menggunakan metode *enzyme-linked immunosorbent assay* (ELISA). Hasil penelitian menunjukkan bahwa kedua vaksin dengan adjuvan secara signifikan meningkatkan kadar antibodi dibandingkan dengan kelompok kontrol. Produksi antibodi mulai terdeteksi sejak minggu kedua setelah vaksinasi, dan mencapai puncaknya pada minggu keempat. Analisis komparatif menunjukkan bahwa Montanide ISA 201 VG menghasilkan respons antibodi yang sedikit lebih tinggi dibandingkan dengan Montanide Gel 01. Temuan ini mendukung penggunaan vaksin *Streptococcus suis* inaktif dengan adjuvan sebagai strategi pencegahan yang efektif untuk meningkatkan kekebalan pada populasi babi dan mengurangi risiko penularan zoonosis.

Kata kunci: *Streptococcus suis*; vaksin inaktif; adjuvan; ELISA; titer antibodi.

INTRODUCTION

Pigs represent economically valuable livestock and are extensively farmed in Indonesia, particularly in regions where religious, cultural, or customary restrictions on pork consumption do not apply. The high demand for pork, both for daily consumption and religious ceremonies, has driven the rapid expansion of the pig farming industry. However, the increasing pig population also heightens the risk of infectious disease outbreaks, posing significant threats to both animal and human health (Sudiastra & Budaarsa, 2021).

One of the most critical infectious threats in pig farming is *Streptococcus suis* (*S. suis*), a zoonotic pathogen capable of transmission from pigs to humans. In Bali, the consumption of undercooked pork has been associated with an increasing incidence of *S. suis*-induced meningitis (Besung et al., 2019). In pigs, *S. suis* infection manifests as severe clinical symptoms, including anorexia, high fever, joint inflammation, and, in severe cases, mortality, resulting in substantial economic losses (Dutkiewicz et al., 2017). In humans, *S. suis* infection can cause bacterial meningitis, which may lead to fatal outcomes. Epidemiological data from Bali indicate that between 2014 and 2017, 44 confirmed *S. suis* infections were identified among 71 suspected meningitis cases, with 29 isolates classified as serotype 2 (Susilawathi et al., 2019). Furthermore, between January and May 2023, 50 cases of human *S. suis* infections were recorded, including four fatalities, underscoring the pathogen's significant public health implications (Mds, 2023).

At present, no commercially available vaccine exists to prevent *S. suis* infection, making antibiotics the primary control measure. However, excessive antibiotic use raises concerns regarding antimicrobial resistance (Agopsowicz et al., 2024). Therefore, the development of an inactivated vaccine with an appropriate adjuvant represents a promising strategy for disease prevention (Pramesti et al., 2022). Adjuvants such as Montanide ISA 201 VG and Montanide

Gel 01 have been shown to enhance immune responses and prolong vaccine-induced protection. Specifically, Montanide ISA 201 VG improves injection efficiency, whereas Montanide Gel 01 offers high stability and a greater antigen-carrying capacity (Cui et al., 2024).

The administration of an inactivated *S. suis* vaccine induces antibody production in pigs, with antibody titers in the supernatant—resulting from vaccine formulations with different adjuvants quantified using the Enzyme-Linked Immunosorbent Assay (ELISA). A comparative analysis will be conducted to determine the most effective adjuvant for *S. suis* vaccine formulation based on antibody responses and their persistence in the supernatant (Pramesti et al., 2022).

MATERIALS AND METHODS

Ethical Approval

All experimental procedures in this study were conducted in accordance with applicable animal welfare standards and were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University with approval No. B/181/UN14.2.9/PT.01.04/2024.

Experimental Animals

The pigs used in this study were 1.5 months old. Prior to treatment, the animals underwent a two-week acclimatization period in designated housing under continuous health monitoring. The study was conducted at a pig farm in Bangbang Village, Tembuku District, Bangli Regency, Bali.

Experimental Design

This study employed an experimental approach using a factorial completely andomized design. The first factor consisted of three treatment groups: P1 (control adjuvant only, without antigen), P2 (*S. suis* vaccine with the Montanide ISA 201 VG adjuvant), and P3 (*S. suis* vaccine with the Montanide Gel 01 adjuvant). The second factor was the observation period, spanning five weeks post-vaccination: Week 1 (W1), Week 2 (W2), Week 3 (W3), Week 4 (W4), and Week 5 (W5). Each treatment group consisted of four pigs.

Bacterial Culture

The *S. suis* isolate used in this study was obtained from the Biomedical Laboratory Collection, Faculty of Veterinary Medicine, Udayana University, and designated as strain IIA3, confirmed through Polymerase Chain Reaction (PCR). Five bacterial colonies cultured on Sheep Blood Agar (SBA) were transferred into 500 mL of Tryptone Soy Broth (TSB) and incubated in a shaker incubator at 37°C for 48 hours. Following incubation, bacterial density was standardized to McFarland 0.5. To ensure isolate purity, the bacteria were subcultured on SBA and analyzed microscopically using Gram staining (Besung et al., 2019).

Bacterial Inactivation

The bacterial isolate was transferred into two separate tubes, each containing 50 mL of bacterial suspension, and centrifuged at 5,000 rpm for 10 minutes. The supernatant was discarded, leaving only the bacterial pellet, which was then resuspended in NaCl solution to a final volume

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of 50 mL. The inactivation process was performed in two stages. The first stage involved ultrasonication using an ultrasonicator set at 70% amplitude for 20 minutes, repeated in three cycles. The second stage consisted of heat inactivation at 80°C for 2 hours using an incubator and water bath. To confirm complete inactivation, the bacterial suspension was cultured on Mueller-Hinton Agar (MHA) to check for any remaining viable bacteria (Besung et al., 2019).

Adjuvant Addition

The inactivated *S. suis* isolate was formulated into a vaccine by adding Montanide Gel 01 (SEPPIC, Fairfield, NJ, USA) adjuvant at a composition of 7.5% adjuvant, 42.5% NaCl, and 50% antigen. For the Montanide ISA 201 VG (SEPPIC, Fairfield, NJ, USA) adjuvant, the formulation consisted of 50% adjuvant and 50% antigen. Polysorbate was added to the vaccine candidates, which were then homogenized using a magnetic stirrer at 1,500 rpm for 25 minutes.

Vaccination and Serum Collection

The vaccine was administered via intramuscular injection at a dose of 4 mL per pig. Blood samples (5 mL) were collected weekly from the jugular vein and allowed to clot to obtain serum. The serum was then centrifuged at 5,000 rpm for 10 minutes and stored for subsequent analysis using ELISA.

ELISA Assay

Post-vaccination antibody titers in serum samples were measured using an indirect ELISA (Obradovic et al., 2021). The ELISA procedure began with coating microplate wells with diluted *S. suis* supernatant antigen (1:10) at a volume of 50 µL per well, followed by overnight incubation at 4°C. The wells were then washed three times with PBS-Tween. Blocking was performed using 10% skim milk (100 µL/well) and incubated for 1 hour at room temperature, followed by three additional washes. Serum samples were diluted (1:100), and 1 µL of each sample was added to the wells, incubated for 1 hour at room temperature, and washed again. Next, 50 µL of anti-swine IgG (H+L alkaline phosphatase, Sigma-Aldrich) diluted at 1:1000 was added to each well, incubated for 1 hour, and washed three times. The enzymatic reaction was initiated by adding 50 µL of p-NPP (p-nitrophenyl phosphate) substrate per well and incubating for 15 minutes at room temperature until a color change occurred. Optical density (OD) was measured using an ELISA reader (Jeffery et al., 2024).

Data Analysis

Differences in antibody titers among adjuvant groups and post-vaccination time points were analyzed using analysis of variance (ANOVA) with SPSS (Statistical Product and Service Solutions) IBM version 25. If significant differences were detected, a least significant difference (LSD) test was performed at a 5% significance level ($p < 0.05$).

RESULTS AND DISCUSSION

Results

The ELISA results demonstrated that the *S. suis* vaccine successfully stimulated an immune response in pigs by inducing antibody production, as shown in Figure 1. Both types of adjuvants, Montanide ISA 201 VG and Montanide Gel 01, exhibited a weekly increase in antibody titers. The rise in antibody titers became detectable in the second week, peaked in the fourth week, and remained stable in the fifth week. Based on the average antibody titers, the group receiving the vaccine with Montanide ISA 201 VG had the highest mean value (0.569),

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followed by the Montanide Gel 01 group (0.563), while the control group had the lowest mean value (0.449).

Further analysis using ANOVA revealed that vaccination significantly increased antibody titers ($p < 0.05$) in pigs receiving *S. suis* vaccines with different adjuvants. Additionally, the weekly increase in antibody titers also showed a significant difference, as presented in Table 1. A post-hoc Least Significant Difference (LSD) test indicated that vaccines formulated with Montanide ISA 201 VG and Montanide Gel 01 significantly enhanced antibody titers against *S. suis* compared to the control group ($p < 0.05$). However, no significant difference was observed between the Montanide ISA 201 VG and Montanide Gel 01 vaccine groups ($p > 0.05$), as shown in Table 2. The weekly increase in antibody titers was also statistically significant ($p < 0.05$). The antibody titers in the first week were significantly lower compared to the second, third, and fourth weeks. However, no significant difference was observed between the fourth and fifth weeks ($p > 0.05$), as presented in Table 3.

The effect of time on antibody formation in pigs vaccinated with *S. suis* was found to be substantial. In the control group (P1), the increase in antibody titers was not statistically significant ($p > 0.05$), with a regression equation of $Y = 26.0036$ and a determination coefficient (R^2) of 0.01. In contrast, the P2 group (Montanide ISA 201 VG) exhibited a significant increase in antibody titers ($p < 0.05$), with a regression equation of $Y = 49.774$ and an R^2 value of 0.844. Similarly, the P3 group (Montanide Gel 01) also showed a significant increase ($p < 0.05$), with a regression equation of $Y = 48.202$ and an R^2 value of 0.810.

Discussion

The results of this study indicate that the inactivated *Streptococcus suis* vaccine formulated with Montanide ISA 201 VG and Montanide Gel 01 adjuvants significantly increased antibody titers compared to the control group. Figure 1 shows that the rise in antibody titers became detectable in the second week, peaked in the fourth week, and stabilized in the fifth week. This pattern aligns with the findings of Pramesti et al. (2022), who reported that the primary immune response to vaccination develops within 7–14 days post-injection, with a peak response occurring around the fourth week. The stabilization of antibody titers after the fourth week suggests that adjuvanted vaccines may extend the duration of protection against *S. suis* infection compared to the control group, which exhibited lower efficacy.

A two-way ANOVA analysis (Table 1) demonstrated that both the vaccine treatment and observation time had significant effects on antibody titer increases ($p < 0.05$). This confirms that vaccination with inactivated *S. suis* combined with adjuvants effectively stimulates a stronger immune response compared to non-vaccination. Additionally, the adjuvant factor had a significant impact ($p < 0.05$), indicating that vaccines with adjuvants provide a more robust immunostimulatory effect than those without adjuvants. Adjuvants enhance antigen presentation to the immune system, thereby optimizing the antibody response and prolonging its duration (Reed et al., 2013). Furthermore, the interaction between adjuvant type and observation time was also significant ($p < 0.05$), suggesting that vaccine efficacy is influenced by the post-injection duration.

Further analysis using the Least Significant Difference (LSD) test on the vaccine treatments (Table 2) revealed that the groups receiving Montanide ISA 201 VG and Montanide Gel 01 showed significantly higher antibody titers compared to the control group ($p < 0.05$). However, the difference between the two adjuvant types was not statistically significant ($p > 0.05$), indicating that both ISA 201 VG and Gel 01 were similarly effective in enhancing antibody titers. These findings are consistent with Suartha et al. (2011), who reported that oil- and gel-based adjuvants could increase antibody titers up to twofold compared to non-adjuvanted

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vaccines. Nonetheless, although not statistically significant, the group vaccinated with Montanide ISA 201 VG exhibited slightly higher average antibody titers than the Montanide Gel 01 group, suggesting a potentially greater effectiveness in sustaining the immune response.

The comparison of antibody titers over time (Table 3) revealed a significant increase each week ($p < 0.05$). The antibody titer in the first week was significantly lower than in the subsequent weeks, indicating that the immune system requires time to recognize the antigen and develop a primary immune response. A more pronounced increase was observed from the second to the fourth week, with the peak response occurring in the fourth week. After this peak, antibody titers stabilized, as evidenced by the absence of a significant difference between the fourth and fifth weeks ($p > 0.521$). This finding is consistent with the theory proposed by Murphy & Weaver (2016), which states that after reaching its peak, the humoral immune response enters a plateau phase before gradually declining over time. The stability of antibody titers after the fourth week also suggests that adjuvanted vaccines can sustain the immune response for an extended period, which is crucial for preventing recurrent infections.

Furthermore, regression analysis demonstrated that time had a significant effect on antibody formation, except in the control group. In both vaccines formulated with Montanide ISA 201 VG and Montanide Gel 01, the duration of observation influenced antibody titers. These results align with the findings of Cui et al. (2024), who reported that oil-based adjuvants enhance antigen retention and slow antigen release into the immune system, thereby providing prolonged stimulation compared to non-adjuvanted vaccine formulations.

Overall, the results of this study confirm that inactivated *S. suis* vaccination with adjuvants significantly increases antibody titers compared to the control group, with the peak immune response occurring in the fourth week before stabilizing in the fifth week. The use of Montanide ISA 201 VG and Montanide Gel 01 as adjuvants was proven effective in enhancing and maintaining antibody titers, although ISA 201 VG exhibited a slight advantage in sustaining the immune response for a longer duration. These findings support the use of inactivated vaccines with adjuvants as an effective strategy for preventing *Streptococcus suis* infections and highlight their potential in reducing antibiotic dependence in pig farming (Pramesti et al., 2022).

CONCLUSION AND RECOMMENDATION

Conclusion

The inactivated *Streptococcus suis* vaccine with Montanide ISA 201 VG or Montanide Gel 01 adjuvants significantly increased antibody titers. A significant increase in antibody titers ($p < 0.05$) was observed up to the fourth week.

Recommendation

Further research is needed to evaluate the long-term efficacy of the vaccine.

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REFERENCES

Agopsowicz, H. M., Sparrow, E., Cameron, A. M., Sati, H., Srikantiah, P., Gottlieb, S., Bentsi-Enchill, A., Le Doare, K., Hamel, M., Giersing, B. K., & Hausdorff, W. P. (2024). The role of vaccines in reducing antimicrobial resistance: A review of potential impact of vaccines on AMR and insights across 16 vaccines and pathogens. Vaccine, 42(19), S1–S8. https://doi.org/10.1016/j.vaccine.2024.06.017.

Besung I.N.K., Suarjana I.G.K., Agustina K.K., Winaya I.B.O., Suharsono H., Suwiti N.K., And Mahardika I.G.K., (2019). Isolation and identification of Streptococcus suis from sick pigs in Bali, Indonesia. BMC Research Notes 12(1), 759. https://doi.org/10.1186/s13104-019-4826-7.

Cui, Y., Ho, M., Hu, Y., & Shi, Y. (2024). Vaccine adjuvans: current status, research and development, licensing, and future opportunities. In Journal of Materials Chemistry B (Vol. 12, Issue 17, pp. 4118–4137). Royal Society of Chemistry. https://doi.org/10.1039/d3tb02861e.

Dutkiewicz, J., Sroka, J., Zając, V., Wasiński, B., Cisak, E., Sawczyn, A., Kloc, A., and Wójcik-Fatla, A. (2017). 'Streptococcus suis: A Re-Emerging Pathogen Associated with Occupational Exposure to Pigs or Pork Products', Annals of Agricultural and Environmental Medicine, 24(4), 683-695. doi: https://doi.org/10.26444/aaem/79813.

Mds. (2023). Hingga Mei, 50 Kasus Meningitis Babi di Bali, Warga Diimbau Tak Konsumsi Daging Mentah. Nusa Bali Com.

Murphy K, Weaver C. 2016. Janeway's Immunobiology. 9th ed. Garland Science.

Obradovic, M. R., Corsaut L., Dolbec D., Gottschalk M., Segura M. 2021. Experimental evaluation of protection and immunogenicity of Streptococcus suis bacterin-based vaccines formulated with different commercial adjuvans in weaned piglets. Veterinary Research, 52: 1-16. https://doi.org/10.1186/s13567-021-01004-x

Pramesti, P. D., Mahardika, I. G. N. K., & Besung, I. N. K. (2022). Titer Antibodi Primer Terhadap Streptococcus suis pada Tikus Putih Yang Divaksin Dengan Adjuvan Berbeda. Buletin Veteriner Udayana, 363. https://doi.org/10.24843/bulvet.2022.v14.i04.p08.

Reed, S. G., Orr, M. T., & Fox, C. B. (2013). Key roles of adjuvans in modern vaccines. In Nature Medicine (Vol. 19, Issue 12, pp. 1597–1608). https://doi.org/10.1038/nm.3409 .

Sudiastra, I. W. & Budaarsa, K. (2021). Eksistensi Babi Bali Melalui Usaba Sumbu di Desa Adat Timbrah Kabupaten Karangasem. Majalan Ilmiah Peternakan. 24 (1), 42-49.

Susilawathi, N.M., Tarini, N.M.A., Fatmawati, N.N.D., Mayura, P.I.B., Suryapraba, A.A.A., Subrata, M., Sudewi, A.A.R., Mahardika, G.N.K., (2019). Streptococcus suis-Associated Meningitis, Bali, Indonesia, 2014-2017. Emerg Infect Dis 25(12): 2235-2242. https://doi.org/10.3201/eid2512.181709

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Table

Table 1. Statistical Results of Two-Way ANOVA for Antibody Titers

Source	F	Sig.
Corrected Model	28.830	<0.001
Intercept	13321.279	<0.001
Adjuvant	73.005	<0.001
Week	43.229	<0.001
adjuvant * Week	10.587	<0.001

Table 2. Least Significant Difference (LSD) Test Results for Different Treatments on Antibody Titers

(I) Vaccine Treatment	(J) Vaccine Treatment	Mean Difference	Sig.
Control	Adjuvant Montanide ISA201	-0.11970*	<0.001
	Adjuvant Montanide Gel 01	-0.11425*	<0.001
Adjuvant Montanide ISA 201	Adjuvant Montanide Gel 01	0.00545	0.629

Table 3. Results of LSD Test Between Time Points on Antibody Titer.

(I) Week treatment	(J) Week treatment	Mean Difference	Sig.
W1	W2	-0.05642*	<0.001
	W3	-0.09467*	<0.001
	W4	-0.15158*	<0.001
	W5	-0.16092*	<0.001
W2	W3	-0.03825*	0.011
	W4	-0.09517*	<0.001
	W5	-0.10450*	<0.001
W3	W4	-0.05692*	<00.001
	W5	-0.06625*	<0.001
W4	W5	-0.00933	0.521

Figure

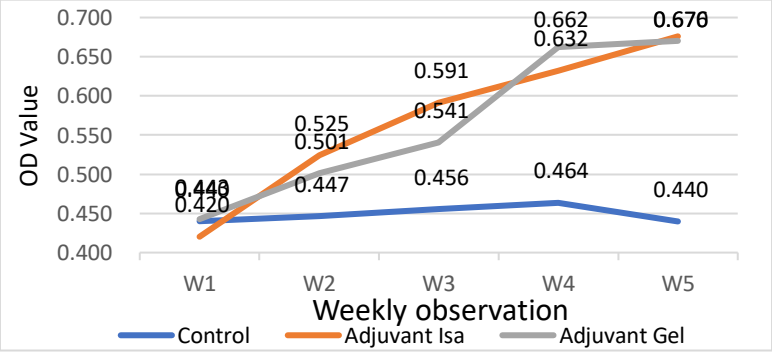

Figure 1. Graph of Significant Weekly Increase in Antibody Titers with the Addition of Various Adjuvants

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