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EFFECT OF *STREPTOCOCCUS SUIS* VACCINES WITH DIFFERENT ADJUVANTS ON PRIMARY ANTIBODY TITERS IN MICE DETECTED BY ELISA TEST USING SUPERNATAN ANTIGENS

Pengaruh Vaksin *Streptococcus suis d*engan Adjuvan Berbeda Terhadap Titer Antibodi Primer pada Mencit Yang Dideteksi dengan Uji Elisa Menggunakan Antigen Supernatan

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Abstract

Streptococcus suis is a zoonotic pathogen that causes infection in pigs and has the potential to be transmitted to humans. One of the effective strategies in the prevention of this infection is vaccination, where adjuvant selection plays an important role in enhancing the immune response. This study aimed to evaluate the effect of using different adjuvants in *S. suis* vaccine on primary antibody titers in mice, measured using ELISA assay with supernatant antigen. This study used a factorial complete randomized design with two factors, namely adjuvant type and observation time. Serum samples were collected weekly for four weeks for antibody titer analysis. The results showed that the vaccine with Montanide ISA 201 VG adjuvant produced significantly higher antibody titers compared to the vaccine using Montanide Gel 01 adjuvant and the control group ($P < 0.05$). The conclusion of this study is

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that Montanide ISA 201 VG adjuvant is more effective in enhancing primary immune responses than Montanide Gel 01, so it has the potential to be used in the development of a more optimal *S. suis* vaccine.

Keywords: *Streptococcus suis*, adjuvant, ELISA test, vaccine, serum

Abstrak

Streptococcus suis merupakan patogen zoonosis yang menyebabkan infeksi pada babi dan berpotensi menular ke manusia. Salah satu strategi efektif dalam pencegahan infeksi ini adalah vaksinasi, di mana pemilihan adjuvan memegang peran penting dalam meningkatkan respons imun. Penelitian ini bertujuan untuk mengevaluasi pengaruh penggunaan adjuvan yang berbeda dalam vaksin *S. suis* terhadap titer antibodi primer pada mencit, yang diukur menggunakan uji ELISA dengan antigen supernatan. Penelitian ini menggunakan Rancangan Acak Lengkap Faktorial dengan dua faktor, yaitu jenis adjuvan dan waktu pengamatan. Sampel serum dikoleksi setiap minggu selama empat minggu untuk analisis titer antibodi. Hasil penelitian menunjukkan bahwa vaksin dengan adjuvan Montanide ISA 201 VG menghasilkan titer antibodi yang signifikan lebih tinggi dibandingkan dengan vaksin yang menggunakan adjuvan Montanide Gel 01 maupun kelompok kontrol ($P < 0,05$). Kesimpulan dari penelitian ini adalah bahwa adjuvan Montanide ISA 201 VG lebih efektif dalam meningkatkan respons imun primer dibandingkan Montanide Gel 01, sehingga berpotensi digunakan dalam pengembangan vaksin *S. suis* yang lebih optimal.

Kata kunci: *Streptococcus suis*, adjuvant, uji ELISA, vaksin, serum

INTRODUCTION

Streptococcus suis is a pathogenic bacterium that causes serious infections in pigs and has zoonotic potential, so it can be transmitted to humans (Segura, 2020). *S. suis* infection can cause various clinical manifestations, including meningitis, septicemia, arthritis, and even death in animals and humans (Pramesti *et al*., 2022). In Indonesia, particularly in Bali, cases of *S. suis* infection have been reported sporadically, with an increasing trend in recent years (Tarini *et al*., 2022).

One of the main strategies in the prevention of these infections is vaccination, which has been shown to be effective in reducing disease incidence (Hussain *et al*., 2018). However, the success of vaccines relies heavily on the selection of adjuvants that can enhance the immune response to the administered antigen (Pulendran & Ahmed, 2011). Adjuvants play a role in amplifying, prolonging and modulating the immune response, thereby increasing the effectiveness of vaccine protection (Reed *et al*., 2013).

Various types of adjuvants have been developed to increase the effectiveness of vaccines, including Montanide ISA 201 VG and Montanide Gel 01. These two adjuvants have different mechanisms of action in triggering the immune response, thus providing varying levels of protection against the target pathogen (Obradovic *et al*., 2021). Therefore, this study aimed to evaluate the effect of using different adjuvants in *S. suis* vaccines on primary antibody titers in mice, as measured using an ELISA assay with supernatant antigen.

The results of this study are expected to contribute to the development of more effective *S. suis* vaccination strategies, thereby reducing the risk of spreading infection in both animals and humans.

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RESEARCH METHODS

Ethical Approval

Based on the research of the Animal Ethics Commission team of Faculty of Veterinary Medicine, Udayana University on the documents submitted. The research procedures are in accordance with the principles of use and principles of animal welfare. And this research already has a Certificate of Animal Ethics Approval with number: B/178/UN14.2.9/PT.01.04/2024.

Object of Research

This study used blood serum of mice (*Mus musculus*) that had been vaccinated with *S. suis* containing different adjuvants. The mice used were two months old with a body weight of about 20-30 grams.

Research Design

This study used a completely randomized factorial design with two main factors. The first factor was the type of vaccination, which consisted of three treatment groups: Adjuvant control without antigen (A1), vaccine with Montanide ISA 201 VG adjuvant (A2), and vaccine with Montanide Gel 01 adjuvant (A3). The second factor was the time of antibody titer measurement: Before vaccination (W1), one week after vaccination (W2), two weeks after vaccination (W3), and three weeks after vaccination (W4).

Research Variables

The variables in this study are categorized into three groups: independent, dependent, and control variables. The independent variables consist of the type of adjuvant used and the observation time. The dependent variable is the primary antibody titer, which is measured using an ELISA test. Meanwhile, the control variables include factors that are kept constant throughout the experiment to ensure consistency, such as the type of mice, environmental conditions, and data analysis methods. By controlling these variables, the study aims to minimize external influences and accurately assess the relationship between adjuvant type, observation time, and antibody production.

Research Procedure

The *S. suis* isolate used was obtained from the Biomedical Laboratory field isolate collection, Faculty of Veterinary Medicine, Udayana University, with code IIA3. This isolate has been confirmed using PCR and cultured in Tripty Soy Broth (TSB). A total of five *S. suis* colonies were inoculated into two tubes containing 500 ml of TSB each, then incubated in a shaker incubator for 48 hours. After that, the number of bacteria was counted using the McFarland 0.5 standard. Identification was done by observing the growth of colonies on the media. Confirmation of these germs was done by microscopic observation with Gram stain.

Vaccine Manufacturing

Germs were inactivated through sonication and heating. A total of 500 ml TSB containing *S. suis* isolates was transferred into two 50 ml Eppendorf tubes (TAB I and TAB II), then centrifuged for 10 min at 5,000 rpm. The supernatant was discarded, while the precipitate was re-mixed until all TSB was used up, then NaCl was added to reach a volume of 80 ml. The first stage of inactivation was performed by sonication using an ultrasonicator at 70% amplitude for 20 min. Next, heating was carried out at 80°C for 2 hours using an incubator and water bath. Solutions from TAB I and TAB II were then combined and tested for sterility by planting 100 µl of suspension on Mueller Hinton Agar (MHA) incubated for 24 hours at 37°C (Besung *et al*., 2019).

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The antigen solution was mixed with adjuvant in the following ratio: Montanide Gel 01 (15%), NaCl (35%), and antigen (50%). For Montanide ISA 201 VG, the ratio is adjuvant (50%) and antigen (50%). The vaccine candidate was then added polysorbate and stirred with a magnetic stirrer at 1,500 rpm for 25 minutes. As a control, 100% montanide Gel 01 adjuvant without antigen was used.

Vaccination and Serum Collection

The vaccine was given by intraperitoneal injection to mice at a dose of 0.4 ml/head. Blood was collected weekly from the first week to the third week post-vaccination. A total of 1 ml of blood was collected from the orbital sinus using a hematocrit tube to obtain approximately 50 µL of serum. Blood samples were collected in Eppendorf tubes in an inclined position, then centrifuged at 5,000 rpm for 10 min to separate serum from blood clots. The serum obtained was stored in a refrigerator until used in the antibody titer assay using ELISA.

ELISA Test

Antibody titers in the serum of post-vaccination mice were measured using indirect ELISA (Obradovic et al., 2021). The process began with a 10× dilution of *S. suis* supernatant antigen using aquabidest, then mixed with coating buffer and applied to microplate wells (50 µL/well) via pipetting. The plate was incubated overnight at 4°C, then washed three times with PBS-Tween.

Next, blocking was done using 10% skim milk (100 µL/well) and incubated for 1 hour at room temperature, then washed three times. Serum samples were diluted 1:100, then added 1 µL/well, incubated 1 hour at room temperature, and washed again. After that, 50 µL of anti-mouse IgG conjugate (H+L alkaline phosphatase, Sigma-Aldrich) was added at a dilution of 1:1000, incubated for 1 hour, and washed three times. The enzymatic reaction started with the addition of p-NPP substrate (p-nitrophenyl phosphate, 50 µL/well), then incubated for 15 minutes at room temperature until color change occurred. Optical Density (OD) was read using an ELISA reader (Jeffery *et al.*, 2024).

Data Analysis

The data obtained were analyzed using Statistical Product and Service Solutions (SPSS) software version 29. Analysis was performed with Analysis of Variance (ANOVA) to test the effect of treatment on antibody titer. If the results of the analysis showed significant differences ($P < 0.05$), then the Least Significant Difference (LSD) further test at the 5% level was used to compare the treatment groups.

RESULT AND DISCUSSION

Results

The results showed that mice vaccinated with Montanide ISA 201 VG adjuvant had the highest antibody titers, compared to the Montanide Gel 01 and control groups. The graph in Figure 1 confirms that the Montanide ISA 201 VG group showed the highest values, while Table 1 records the highest mean Optical Density (OD) value in this group (0.2758±0.1297), followed by Montanide Gel 01 (0.2220±0.1368), and the control with the lowest OD (0.1532±0.0974).

Two-way ANOVA statistical analysis Table 2 showed significant differences in OD values ($P < 0.001$) based on vaccination treatment, adjuvant type, and post-vaccination observation time. BNT test Table 3 confirmed that the antibody titers of the control group were significantly lower than those of Montanide ISA 201 VG and Montanide Gel 01 ($P < 0.05$), with ISA 201 VG showing the highest titers ($P < 0.05$).

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In the time-based analysis Table 4, antibody titers increased every week, with significant differences between the first, second, third, and fourth weeks ($P < 0.05$). Regression tests (Table 5) showed an increase in antibody titers of $Y = 0.260 + 10.837X$ with $R^2 = 0.339$. Specifically, administration of Montanide ISA 201 VG resulted in an increase of $Y = 0.292 + 8.004X$ ($R^2 = 0.826$), while Montanide Gel 01 was $Y = 0.843 + 7.461X$ ($R^2 = 0.798$).

Discussions

The results of this study indicate that Montanide ISA 201 VG adjuvant is more effective in increasing antibody titers than Montanide Gel 01. Montanide ISA 201 VG has a slower and more controlled antigen release mechanism, allowing for more sustained stimulation of the immune system (Reed et al., 2013). In contrast, Montanide Gel 01 triggered immune responses faster, but for a shorter duration than Montanide ISA 201 VG (Obradovic et al., 2021).

The stronger immune response in the Montanide ISA 201 VG group is thought to be related to increased activation of antigen presenting cells (APCs), which facilitates antigen presentation to B and T cells for specific antibody production (Pulendran & Ahmed, 2011). The increase in antibody titers from week one to week four indicates the development of a good adaptive immune response, with increased antibody production after initial vaccination (Hussain et al., 2018).

Two-way ANOVA statistical analysis confirmed significant differences between treatment groups ($P < 0.05$). A further least significant difference (LSD) test showed that Montanide ISA 201 VG had a significantly higher immune response than Montanide Gel 01 and the control group, confirming its effectiveness as an adjuvant.

These results are in line with previous studies, which stated that oil-based emulsions, such as Montanide ISA 201 VG, are more effective in enhancing immune responses than gel-based adjuvants (Coffman *et al.*, 2010). The stability of the antibody increase until the fourth week suggests that vaccination with Montanide ISA 201 VG has the potential to provide better long-term protection than other adjuvants.

Thus, this study confirmed that Montanide ISA 201 VG is a more optimal adjuvant in *Streptococcus suis* vaccines, providing a scientific basis for the development of more effective vaccines in the future.

CONCLUSION AND RECOMENDATIONS

Conclusions

Inactivated *Streptococcus suis* vaccine with Montanide ISA 201 VG and Montanide Gel 01 adjuvants significantly increased primary antibody titers compared to controls ($P < 0.05$). Antibody titers increased significantly from the first week to the fourth week ($P < 0.05$).

Recommendations

Further research needs to be done on the side effects of vaccine administration and observations need to be carried out longer.

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Table

Table 1. Mean OD (Optical Density) and SD (Standard Deviation) Values of Various Adjuvants.

	Adjuvant Type	
	w/o/w	gel
Average	0.2758	0.222
±SD	0.1297	0.136

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Table 2. Statistical Results of Two-way Anova Test on OD Values

Source	F	Sig.
Corrected Model	25.173	<0.001
Intercept	1030.225	<0.001
Vaccine	29.617	<0.001
Week	60.710	<0.001
Vaccine * Week	5.923	<0.001
Error		
Total		
Corrected Total		

Table 3. Least Significant Difference (LSD) Test Results Between Various Treatments on OD Values

(I) Vaccine Treatment	(J) Vaccine Treatment	Mean Difference	Sig.
Control	Adjuvant ISA 201 VG	-.12629*	<0.001
	Adjuvant Gel 01	-.07250*	<0.001
Adjuvant ISA 201 VG	Control	.12629*	<0.001
	Adjuvant Gel 01	.05379*	0.002
Adjuvant Gel 01	Control	.07250*	<0.001
	Adjuvant ISA 201 VG	-.05379*	0.002

Table 4. Least Significant Difference (LSD) Test Results Between Time and OD Value

(I) Week Treatment	(J) Week Treatment	Mean Difference	Sig.
Week 1	Week 2	-.09844*	<.001
	Week 3	-.18578*	<.001
	Week 4	-.23922*	<.001
Week 2	Week 1	.09844*	<.001
	Week 3	-.08733*	<.001
	Week 4	-.14078*	<.001
Week 3	Week 1	.18578*	<.001
	Week 2	.08733*	<.001
	Week 4	-.05344*	.007
Week 4	Week 1	.23922*	<.001
	Week 2	.14078*	<.001
	Week 3	.05344*	.007

Table 5. Regression Test Results Between Treatment and Time on OD Values

Treatment	Regression Equation	R^2	Sig.
Kontrol	Y=0.260+10.837X	0.339 (33.9%)	0.003
Montanide ISA 201 VG	Y=0.292+8.004X	0.826 (82.6%)	<0.001
Montanide Gel 01	Y=0.843+7.461X	0.798 (79.8%)	<0.001

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Figure

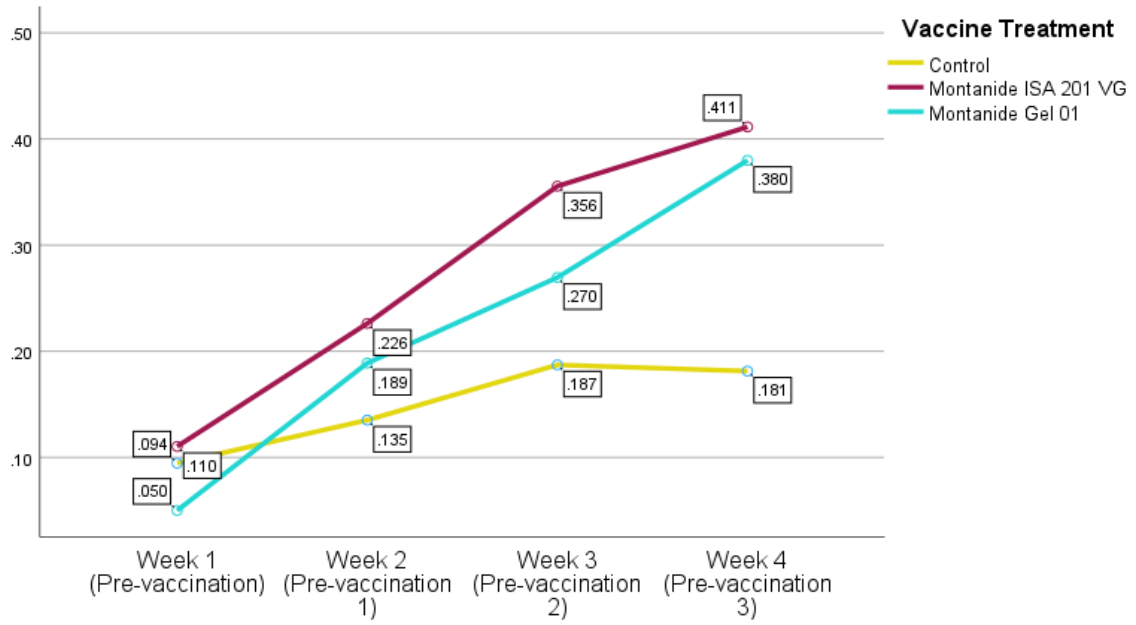

Figure 1. Graphic of weekly increase in OD values with the addition of various adjuvants.