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COMPARISON OF ADJUVANT EFFICACY IN *STREPTOCOCCUS SUIS* VACCINATION IN MICE: SECONDARY ANTIBODY TITER ANALYSIS USING ANTIGEN SUPERNATANT BASED ELISA

Perbandingan Efektivitas Adjuvan Dalam Vaksinasi *Streptococcus suis* Pada Mencit: Analisis Titer Antibodi Sekunder Dengan Elisa Berbasis Supernatan Antigen

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Abstract

Streptococcus suis is a Gram-positive zoonotic bacterium that causes various diseases in pigs, such as meningitis, arthritis, and septicemia, which affect animal health and the economic viability of the livestock industry. Vaccination, supported by appropriate adjuvants, is an effective control strategy to enhance immune responses. This study aimed to evaluate secondary antibody responses in mice vaccinated with *S. suis* antigen using two different adjuvants, Montanide™ ISA 201 VG and Montanide™ Gel 01, and to analyze the dynamics of antibody titer increase. Eighteen mice were divided into three treatment groups: a control group receiving adjuvant without antigen, a vaccine group with Montanide™ ISA 201 VG, and a vaccine group with Montanide™ Gel 01. Vaccination was administered twice, and blood samples were collected weekly for four weeks post-vaccination. Antibody titers specific to *S. suis* supernatant antigen were measured using the ELISA method. Data were analyzed using ANOVA, Least Significant Difference (LSD) test, and regression analysis. The results showed that both adjuvants significantly increased antibody titers compared to the control ($p<0.05$), with a progressive increase in titer levels. Montanide™ ISA 201 VG provided sustained

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stimulation through a depot effect, while Montanide™ Gel 01 elicited an effective immune activation with minimal local reactions. In conclusion, the combination of *S. suis* antigen with both adjuvants significantly enhanced humoral immune responses, supporting the potential of both adjuvants to improve *S. suis* vaccine efficacy. Further research is needed to determine the optimal dose and duration of post-vaccination immune protection.

Keywords: *Streptococcus suis*; vaccine; adjuvant; mice; antibody titer; ELISA

Abstrak

Streptococcus suis adalah bakteri Gram-positif zoonotik penyebab berbagai penyakit pada babi, seperti meningitis, arthritis, dan septikemia, yang berdampak pada kesehatan hewan dan ekonomi industri peternakan. Vaksinasi dengan dukungan adjuvan yang tepat merupakan strategi pengendalian yang efektif untuk meningkatkan respons imun. Penelitian ini bertujuan mengevaluasi respons antibodi sekunder pada mencit yang divaksinasi dengan antigen *S. suis* menggunakan dua adjuvan berbeda, yaitu Montanide™ ISA 201 VG dan Montanide™ Gel 01, serta menganalisis dinamika peningkatan titer antibodi. Sebanyak 18 ekor mencit dibagi menjadi tiga kelompok perlakuan: kontrol berupa adjuvan tanpa antigen, vaksin dengan Montanide™ ISA 201 VG, dan vaksin dengan Montanide™ Gel 01. Vaksinasi dilakukan dua kali, dan sampel darah dikoleksi setiap minggu selama empat minggu pasca-vaksinasi. Titer antibodi spesifik terhadap antigen supernatan *S. suis* diukur menggunakan metode ELISA. Data dianalisis dengan ANOVA, uji Beda Nyata Terkecil (LSD), dan analisis regresi. Hasil penelitian menunjukkan bahwa kedua adjuvan secara signifikan meningkatkan titer antibodi dibandingkan kontrol ($p<0.05$), dengan kecenderungan peningkatan titer yang progresif. Montanide™ ISA 201 VG memberikan stimulasi berkepanjangan melalui efek depot, sementara Montanide™ Gel 01 menghasilkan aktivasi imun efektif dengan reaksi lokal minimal. Disimpulkan bahwa kombinasi antigen *S. suis* dengan kedua adjuvan meningkatkan respons imun humoral secara signifikan, mendukung potensi kedua adjuvan dalam meningkatkan efektivitas vaksin *S. suis*. Penelitian lanjutan diperlukan untuk menentukan dosis optimal dan durasi perlindungan imun pasca-vaksinasi.

Kata kunci: *Streptococcus suis*; vaksin; adjuvant; mencit; titer antibody; ELISA

INTRODUCTION

Streptococcus suis (*S. suis*) is a Gram-positive, zoonotic bacterium recognized as a significant pathogen in both pigs and humans. In pigs, *S. suis* infection can lead to a range of severe diseases, including meningitis, bronchopneumonia, arthritis, pericarditis, polyserositis, and septicemia (Kasianenko and Liu, 2023). These infections not only pose serious threats to animal health but also cause substantial economic losses in the swine industry. In humans, *S. suis* can result in clinical manifestations such as meningitis, high fever, and hearing loss. To date, 35 serotypes of *S. suis* have been identified, with two classified as highly virulent and having strong zoonotic potential (Wayop et al., 2025).

In Indonesia, particularly in Bali Province, the prevalence of *S. suis* infection is notably high and has emerged as an urgent public health issue. The consumption of traditional raw pork-based dishes is suspected to be one of the main routes of bacterial transmission to humans. Confirmed human cases have been reported in several regions, including Tabanan, Denpasar, Gianyar, and Karangasem, with morbidity rates reaching 18.7%, mortality at 8.4%, and a case fatality rate as high as 44.9% (Besung et al., 2019). Furthermore, a recent report identified eight *S. suis*-positive isolates from 30 suspected infection cases in local pigs (Besung et al., 2022), reinforcing the evidence that this pathogen poses a serious threat in the region.

The management of *S. suis* infections currently relies heavily on antibiotic use. However, uncontrolled antibiotic usage has contributed to the emergence of antimicrobial resistance, exacerbating the challenges in controlling this disease in both animals and humans. Consequently, there is a pressing need for more sustainable control strategies, one of which is vaccination. Vaccination not only serves as a preventive measure in animals but also plays a crucial role in reducing antibiotic usage, thereby mitigating the development of antimicrobial resistance (Segura, 2015).

Nevertheless, vaccine development against *S. suis* remains challenging, particularly in terms of achieving effective and long-lasting protection. One promising approach to enhance vaccine-induced immune responses is the use of suitable adjuvants. Montanide™ Gel 01 and Montanide™ ISA 201 VG are two modern adjuvants that have demonstrated the ability to improve vaccine efficacy by stimulating humoral immune responses. Montanide™ ISA 201 VG, for instance, is known for its capability to induce both short- and long-term immunity, while also possessing low viscosity, which facilitates ease of application (Li et al., 2013).

To date, comparative studies on the efficacy of these two Montanide™ adjuvants in vaccine formulations using local *S. suis* isolates from Bali are still limited. In addition, the use of supernatant antigen-based ELISA for evaluating post-vaccination antibody titers in a mouse model offers a practical, sensitive, and efficient method to assess immune responses. Based on the aforementioned background, this study aims to evaluate the efficacy of *S. suis* vaccines formulated with two different adjuvants in stimulating secondary antibody responses in mice, as well as to analyze the dynamics of antibody titers over the observation period. The results of this study are expected to provide a scientific foundation for the development of more effective vaccines to prevent *S. suis* infection in pigs and reduce the risk of zoonotic transmission to humans, particularly in endemic areas such as Bali.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University, following a thorough review of the submitted research protocol. All procedures were deemed to comply with the principles of animal use and welfare, under approval number: B/178/UN14.2.9/PT.01.04/2024.

Experimental Animals

A total of 18 female mice (*Mus musculus*), aged two months, were used in this study. Prior to treatment, all animals underwent a one-week acclimatization period in laboratory cages, during which their health status was monitored regularly to ensure physiological stability.

Experimental Design

This research employed a factorial completely randomized design (CRD) with two main factors. The first factor was the type of vaccine adjuvant treatment: P1: Control group receiving adjuvant without antigen, P2: Vaccination with Montanide™ ISA 201 VG, and P3: Vaccination with Montanide™ Gel 0. The second factor was the sampling period, which consisted of four time points: W1: at the time of the second vaccination, W2: 7 days post-second vaccination, W3: 14 days post-second vaccination, and W4: 21 days post-second vaccination. Each treatment combination was replicated using 6 individual mice.

Bacterial Culture

The *Streptococcus suis* isolate used in this study was obtained from a stock culture maintained at the Biomedical Laboratory, Faculty of Veterinary Medicine, Udayana University, under

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isolate code IIA3. The identity of the isolate had been previously confirmed using Polymerase Chain Reaction (PCR) analysis. For propagation, the isolate was cultured in Tryptic Soy Broth (TSB). Five pure colonies of *S. suis* were each inoculated into two tubes containing 500 mL of TSB and incubated in a shaker incubator for 48 hours at the optimal temperature. Following incubation, the bacterial suspension was adjusted to a uniform concentration using the 0.5 McFarland standard to achieve a consistent bacterial cell density. The culture was then streaked onto Mueller Hinton Agar (MHA) to confirm the purity of the isolate. Colony morphology and Gram staining were performed to verify the microscopic characteristics consistent with *S. suis* identity (Winaya et al., 2023).

Bacterial Inactivation Procedure

The inactivation of *S. suis* was performed in two stages sonication and heat treatment—to obtain inactivated antigen for vaccine formulation (Obradovic et al., 2021). A total of 500 mL of *S. suis* culture grown in TSB was gradually transferred into two 50 mL centrifuge tubes labeled TAB I and TAB II. The tubes were centrifuged at 5,000 rpm for 10 minutes to separate the bacterial pellet from the supernatant. The supernatant was discarded, and the pellet was resuspended in sterile NaCl solution. This process was repeated until the entire culture volume had been processed. The final bacterial pellet was resuspended in sterile NaCl to a total volume of 50 mL.

The first stage of inactivation involved sonication using an ultrasonic processor set at 70% amplitude for 20 minutes to mechanically disrupt the bacterial cell walls. The resulting suspension was then subjected to heat inactivation by incubation in a water bath at 80°C for 2 hours to ensure complete inactivation through protein and enzyme denaturation. After the inactivation process, the contents of TAB I and TAB II were combined into a single homogeneous solution. To verify successful inactivation, a portion of the suspension was re-cultured on Mueller Hinton Agar (MHA) and incubated at 37°C for 24 hours. The absence of bacterial colony growth on MHA was used as an indicator of complete inactivation (Besung et al., 2019; Rumapea et al., 2022).

Vaccine Formulation

Streptococcus suis vaccine formulations were prepared using two types of adjuvants: Montanide™ Gel 01 and Montanide™ ISA 201 VG (SEPPIC, Fairfield, NJ, USA). The formulation using Montanide™ Gel 01 consisted of 15% adjuvant, 35% sterile NaCl solution, and 50% inactivated *S. suis* antigen. Meanwhile, the Montanide™ ISA 201 VG-based vaccine was prepared as a water-in-oil emulsion at a 1:1 ratio, composed of 50% adjuvant and 50% antigen. Each vaccine candidate was supplemented with polysorbate as a stabilizing agent to enhance emulsion homogeneity and stability. The homogenization process was carried out using a magnetic stirrer at 1,500 rpm for 25 minutes until a stable and uniform emulsion was achieved. For the control group, a formulation containing adjuvant only (without antigen) was used.

Vaccination Procedure

Following a one-week acclimatization period, the mice were vaccinated subcutaneously with a 0.4 mL dose per animal. The vaccination was administered twice: a primary vaccination on day 1 of treatment and a booster vaccination four weeks later to stimulate a secondary immune response. Throughout the experimental period, the health status of the mice was regularly monitored to ensure the absence of local or systemic adverse effects due to vaccination.

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Serum Collection and Preparation

Blood samples were collected weekly for four weeks following the booster vaccination, with approximately 1 mL drawn from each mouse at each time point. Blood collection was performed using capillary tubes via the orbital sinus or retro-orbital plexus technique, targeting the medial canthus the space between the eyeball and the optic foramen. This procedure was conducted carefully to minimize stress and injury to the animals.

The collected blood was placed into sterile Eppendorf tubes and allowed to stand at room temperature in a slanted position for 30–60 minutes to facilitate coagulation and serum separation. Samples were then centrifuged at 5,000 rpm for 10 minutes to separate the serum from blood cellular components. The resulting serum was aseptically transferred into sterile microtubes and stored at 4°C for no longer than 24 hours prior to analysis. The serum samples were subsequently used for the measurement of secondary antibody titers using the indirect Enzyme-Linked Immunosorbent Assay (ELISA) method (Rumapea et al., 2022).

Measurement of Antibody Titers Using the Indirect ELISA Method

Secondary antibody titers in mouse serum were measured using the indirect Enzyme-Linked Immunosorbent Assay (ELISA) method. *S. suis* supernatant antigen was diluted in coating buffer (pH 9.6) at a ratio of 1:250. A volume of 50 µL of the antigen solution was added to each well of a flat-bottom 96-well microtiter plate. The plate was then sealed and incubated overnight at 4°C to allow the antigen to adsorb to the surface of the wells.

Following incubation, the plate was washed three times with phosphate-buffered saline containing 0.05% Tween-20 (PBS-T) to remove unbound antigen. Blocking was carried out by adding 57 µL of blocking solution (0.25 g of skim milk in 5 mL PBS-T) to each well to cover non-specific binding sites. The plate was incubated for 1 hour at room temperature, followed by another three washes with PBS-T. Mouse serum samples were diluted in PBS-T, and 2 µL of serum was added to each well containing 50 µL of PBS-T.

The plate was then incubated for 1 hour at room temperature to allow specific antibodies in the serum to bind to the plate-bound antigens. After incubation, the plate was washed again three times with PBS-T. Next, 50 µL of alkaline phosphatase-conjugated anti-mouse IgG (Sigma-Aldrich) was added to each well and incubated for 1 hour at room temperature. The plate was washed three more times to remove unbound conjugates. Then, 50 µL of p-nitrophenyl phosphate (pNPP) substrate (Sigma) was added to each well, and the plate was incubated for 15–30 minutes at room temperature until a yellow color developed as a result of the enzymatic reaction. Finally, absorbance was measured using an ELISA reader at a wavelength of 400 nm to determine the optical density (OD) values, which reflect the concentration of *S. suis* specific antibodies in the serum samples (Jeffery et al., 2024).

Data Analysis

Differences in antibody titers among adjuvant groups, as well as the dynamics of antibody titer increases in response to the supernatant antigen, were statistically analyzed using IBM SPSS (Statistical Package for the Social Sciences) version 29. The data were analyzed based on a completely randomized factorial design. If significant differences were detected, the analysis was followed by a Least Significant Difference (LSD) test at a 5% significance level ($P < 0.05$).

RESULTS AND DISCUSSION

Results

Serum samples were collected weekly, starting from the first week (W1) to the fourth week (W4) following the booster vaccination. The collected sera were analyzed using an indirect

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Enzyme-Linked Immunosorbent Assay (ELISA) to measure specific antibody titers against *S. suis* antigen. Weekly antibody titer data from each treatment group including the control group (P1), the group vaccinated with Montanide™ ISA 201 VG adjuvant (P2), and the group vaccinated with Montanide™ Gel 01 adjuvant (P3) are presented in Figure 1.

The results showed that both P2 and P3 groups exhibited significantly higher antibody titers compared to the control group (P1). Interestingly, in the first week (W1), the P3 group demonstrated a lower antibody response than the P2 group. However, a more consistent increase in antibody titers was observed in the P3 group over the following weeks. By the fourth week (W4), this group showed the highest antibody titers among all groups.

Statistical analysis of the antibody titer data was conducted using analysis of variance (ANOVA), and the results are presented in Table 1. The analysis revealed that the vaccination treatment had a significant effect on antibody titers ($p<0.05$). Similarly, the observation time (weeks 1 to 4) also significantly affected antibody titers ($p>0.05$). A significant interaction was also observed between the vaccine treatment and observation time ($p>0.05$), indicating that the pattern of antibody titer increase was influenced by the type of vaccine treatment over time.

Post-hoc analysis using the Least Significant Difference (LSD) test, as shown in Table 2, indicated that both P2 and P3 groups had significantly higher antibody titers than the control group ($p< 0.05$). However, no significant difference was observed between the P2 and P3 groups ($p>0.05$), although descriptively, the P3 group showed higher antibody titers in the fourth week. Weekly comparisons using the LSD test (Table 3) demonstrated that antibody titers increased significantly from week 1 to week 4 ($p<0.05$), except between week 2 and week 3, where no statistically significant difference was found ($p>0.05$). Regression analysis was conducted to evaluate the relationship between observation time and antibody titers within each treatment group. The results showed that the increase in antibody titers in the control group (P1) was not statistically significant ($p>0.05$). In contrast, the P2 group exhibited a significant relationship ($p<0.05$) with the regression model $Y = -2.781 + 10.205X$ ($R^2 = 0.650$), while the P3 group also showed a significant relationship ($p>0.05$) with the regression model $Y = -1.879 + 8.307X$ ($R^2 = 0.728$). The high R^2 values indicate that time was a substantial factor explaining the variation in antibody titer increases in both vaccinated groups

Discussion

As shown in Figure 1, antibody titers were markedly low in the control group (adjuvant without antigen) compared to the vaccinated groups. This result indicates that the *Streptococcus suis* antigen was capable of stimulating a humoral immune response, as evidenced by the induction of specific antibody titers. This immunogenicity is attributed to structural components of *S. suis*, such as surface-associated proteins, capsular polysaccharides, and virulence factors like enzymes and toxins, including suilysin. These proteins and polysaccharides are recognized as non-self by the host immune system, thereby activating B cells (Breitfelder et al., 2024).

Upon recognition by antigen-presenting cells (APCs), such as macrophages and dendritic cells, the antigen is processed and presented on the cell surface via MHC class II molecules. This presentation activates CD4+ T-helper cells, which then secrete cytokines that promote B cell proliferation, differentiation, and the production of specific antibodies against the *S. suis* antigen. These antibodies play essential roles in neutralizing toxins, opsonizing bacteria for phagocytosis, and activating the complement system for bacterial lysis (Fantoni et al., 2024).

As shown in Tables 1 and 2, both vaccine formulations with Montanide™ ISA 201 VG and Montanide™ Gel 01 adjuvants significantly enhanced antibody titers. The presence of adjuvants in vaccine formulations contributes to a stronger immune response. Adjuvants such

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as Montanide™ ISA 201 VG and Montanide™ Gel 01 can prolong antigen exposure, recruit immune cells to the injection site, and enhance antigen presentation, thereby producing a more robust and sustained humoral response. Thus, the ability of the *S. suis* antigen to stimulate antibody production depends not only on its intrinsic immunogenic properties but also on efficient antigen presentation by APCs and the immunostimulatory effects of the adjuvants used (Chen and Missiakas, 2024).

Adjuvants are crucial components of vaccine formulations due to their ability to amplify the immune response. In the case of *S. suis* vaccines, the use of Montanide™ ISA 201 VG and Montanide™ Gel 01 has been shown to significantly enhance antibody generation. These adjuvants work by prolonging antigen exposure to the immune system and enhancing the activation of immune cells, thereby optimizing the humoral immune response (El-Bagoury et al., 2015).

Montanide™ ISA 201 VG is a water-in-oil-in-water (W/O/W) emulsion-based adjuvant that creates a depot effect at the injection site, allowing for slow antigen release. This extended release provides ample time for the immune system to recognize and respond to the antigen. Moreover, ISA 201 VG enhances the recruitment and activation of APCs such as macrophages and dendritic cells, which in turn stimulate T and B cell responses. This activation leads to B cell differentiation into plasma cells, producing large amounts of specific antibodies (Rumapea et al., 2022).

In contrast, Montanide™ Gel 01 is a carbomer-based gel adjuvant that also retains the antigen at the injection site and facilitates its gradual release. Although it does not form emulsions like ISA 201 VG, its gel matrix stimulates local cytokine and chemokine production, attracting immune cells to the site of injection (GuzmÃ et al., 2021). This stimulation enables Gel 01 to effectively enhance antibody titers while producing minimal local side effects (SE et al., 2020). The combination of a purified antigen and the appropriate adjuvant is therefore essential for generating a strong and long-lasting immune response (Alsaid et al., 2020).

The primary differences between Montanide™ ISA 201 VG and Montanide™ Gel 01 lie in their physical properties, mechanisms of action, and the type of immune response they elicit. ISA 201 VG is an emulsion-based adjuvant (W/O/W), whereas Gel 01 is a carbomer-based gel. These differences influence how the antigen is presented to the immune system and which immunological pathways are activated (Taghizadeh et al., 2023).

Montanide™ ISA 201 VG forms an antigen depot at the injection site, enabling slow and sustained antigen release. This results in prolonged stimulation of immune cells, particularly APCs, thereby eliciting both humoral and cellular immune responses. ISA 201 VG also tends to activate both Th1 and Th2 helper T lymphocytes, promoting IgG antibody production and cytotoxic T cell responses. These properties make ISA 201 VG highly effective in vaccines targeting pathogens requiring both humoral and cellular immunity (Varma et al., 2022).

Conversely, Montanide™ Gel 01 predominantly stimulates the humoral immune response (Obradovic et al., 2021). Its gel matrix stabilizes the antigen at the injection site without forming an emulsion. While it also releases antigen gradually, the duration is relatively shorter than ISA 201 VG. Gel 01 is more biocompatible and induces lower local inflammation, making it suitable for vaccine formulations that prioritize safety while still producing a significant antibody response. Therefore, the choice between these adjuvants should be based on the nature of the antigen, the intended vaccination goal, and the desired immune profile.

Table 3 shows that *S. suis* vaccines formulated with either Montanide™ ISA 201 VG or Montanide™ Gel 01 consistently increased antibody titers on a weekly basis. The consistent

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weekly increase in antibody titers after vaccination demonstrates the ability of the formulated *S. suis* vaccines to induce an effective adaptive immune response. Following the primary immunization, the murine immune system begins to recognize *S. suis* antigens as foreign, triggering activation of immune cells such as dendritic cells and macrophages to process and present the antigen to T and B lymphocytes. This process leads to the formation of primary antibodies, detectable through the increased titers observed from the first week post-vaccination.

In the subsequent weeks, particularly after the booster dose, a secondary immune response is triggered, characterized by a rapid and heightened surge in antibody titers. This indicates the establishment of immunological memory from the initial vaccination. Montanide™ ISA 201 VG, through its depot effect and sustained antigen release, supports prolonged antigenic stimulation, thereby extending B cell activity for antibody production. Meanwhile, Montanide™ Gel 01, despite its shorter duration of action, still provides strong stimulation to B cells, particularly in promoting the production of IgG antibodies specific to *S. suis* antigens (Ali et al., 2024).

The observed weekly increase in antibody titers reflects the effectiveness of the vaccine formulation in eliciting a progressive immune response. Both adjuvants play critical roles in maintaining continuous immune system stimulation following antigen inoculation (Bagherzadeh et al., 2025). This response is vital in the context of protection against *S. suis* infection, as the antibodies produced not only serve as immune markers but also function in pathogen neutralization, opsonization, and complement system activation. Thus, the use of Montanide™ ISA 201 VG and Gel 01 supports the efficacy of the vaccine in sustainably stimulating antibody production, which is a key indicator in the development of protective vaccines (Wang et al., 2024).

CONCLUSION AND RECOMMENDATION

Conclusion

Vaccination with *S. suis* antigens combined with Montanide™ ISA 201 VG or Montanide™ Gel 01 adjuvants significantly increased specific antibody titers in mice. Both adjuvants enhanced the humoral immune response, with ISA 201 VG providing prolonged stimulation through its depot effect, while Gel 01 offered effective immune activation with minimal local reactions. These results highlight the potential of both adjuvants to improve the efficacy of *S. suis* vaccines.

Recommendation

Further studies are recommended to evaluate the in vivo efficacy of *S. suis* vaccines using varying antigen concentrations to determine the optimal dose for inducing a stronger and more consistent humoral immune response. Long-term monitoring of post-vaccination antibody titers is also needed to assess the decline in antibody levels and to determine the effective duration of immune protection.

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Competing interests

The authors have disclosed no conflicts of interest.

Ethical considerations

The authors confirm that all authors have reviewed and submitted the manuscript to this journal for the first time. Additionally, all authors checked the originality of data and sentences via plagiarism checkers.

Availability of data and materials

The original data presented in the study are included in the article. For inquiries, please contact the corresponding author

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Figure

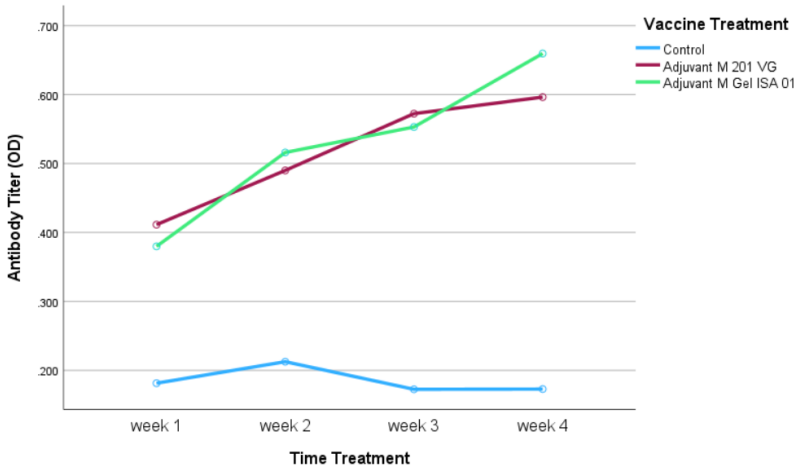

Figure 1. Secondary Antibody Titers in Mice Weekly in the Control Group (P1), Montanide™ ISA 201 VG Adjuvant Group (P2), and Montanide™ Gel 01 Adjuvant Group (P3)

Table

Table 1. Results of the Analysis of Variance for Secondary Antibody Titers in Mice Weekly in the Control Group (P1), Montanide™ ISA 201 VG Adjuvant Group (P2), and Montanide™ Gel 01 Adjuvant Group (P3)

Source	F	Sig.
Corrected Model	42.750	<0.001
Intercept	2587.253	<0.001
Adjuvant	195.110	<0.001
Week	15.764	<0.001
adjuvant * Week	5.456	<0.001

Table 2**.** Results of the LSD Test for Secondary Antibody Titers Against *S. suis* with Different Adjuvants

Vaccine Treatment	Vaccine Treatment	Mean Difference	Sig.
Control	Adjuvan M ISA 201 VG	-0.33271*	<0.001
	Adjuvan M Gel 01	-0.34229*	<0.001
Adjuvan M ISA 201 VG	Adjuvan M Gel 01	-0.00958	0.629

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Table 3. Results of the LSD Test for Secondary Antibody Titers Against *S. suis* Vaccine Weekly

Week treatment	Week treatment	Mean Difference	Sig.
W1	W2	-0.08200*	<0.001
	W3	-0.10844*	<0.001
	W4	-0.15206*	<0.001
W2	W3	-0.02644	0.250
	W4	-0.07006*	0.003
W3	W4	0.10844*	<0.001

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