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EFFECTS OF DIFFERENT MONTANIDE ADJUVANTS ON TOTAL ERYTHROCYTE COUNTS IN PIGLETS VACCINATED WITH A LOCAL *STREPTOCOCCUS SUIS* STRAIN

Pengaruh Berbagai Adjuvan Montanide™ terhadap Jumlah Total Eritrosit pada Anak Babi yang Divaksinasi dengan *Streptococcus suis* Strain Lokal

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Abstract

Streptococcus suis is an important zoonotic pathogen in pigs that causes substantial economic losses and public health concerns. Vaccination using local bacterial strains is considered a promising approach to improve antigenic compatibility and protective immune responses. However, information regarding the effects of different vaccine adjuvants on total erythrocyte counts remains limited. This study aimed to evaluate total erythrocyte counts in piglets vaccinated with an inactivated local *S. suis* strain formulated with different Montanide™ adjuvants. A total of 12 eight-week-old male Landrace piglets were randomly divided into three groups: control (sterile 0.9% physiological saline), vaccine with Montanide™ ISA 201 VG, and vaccine with Montanide™ Gel 01. Vaccination was administered intramuscularly twice at a 28-day interval. Clinical observations, body weight measurements, and blood sampling were performed weekly for nine weeks. Total erythrocyte counts were analyzed using a VetScan HM5 hematology analyzer, and data were evaluated using repeated measures ANOVA. No significant differences in total erythrocyte counts were observed among treatment groups ($p > 0.05$). However, observation time significantly affected erythrocyte counts ($p < 0.05$), with temporary increases occurring during weeks 3 to 5. Overall erythrocyte values remained within normal physiological ranges. In conclusion, inactivated *S. suis* vaccines formulated with Montanide™ ISA 201 VG and Montanide™ Gel 01 did not significantly affect total erythrocyte counts in Landrace piglets and were well tolerated under the conditions of this study.

Keywords: *Streptococcus suis*, erythrocyte, Montanide™, piglets, vaccine safety

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Abstrak

Streptococcus suis merupakan bakteri zoonotik penting pada babi yang menyebabkan kerugian ekonomi dan masalah kesehatan masyarakat. Penggunaan vaksin berbasis strain lokal dianggap sebagai pendekatan yang menjanjikan untuk meningkatkan kesesuaian antigenik dan respons imun protektif. Namun, informasi mengenai pengaruh berbagai jenis adjuvan vaksin terhadap jumlah total eritrosit masih terbatas. Penelitian ini bertujuan untuk mengevaluasi jumlah total eritrosit pada anak babi yang divaksinasi dengan *S. suis* strain lokal inaktif menggunakan formulasi adjuvan Montanide™ yang berbeda. Sebanyak 12 ekor anak babi Landrace jantan berumur delapan minggu dibagi secara acak menjadi tiga kelompok, yaitu kontrol (saline fisiologis 0,9% steril), vaksin dengan Montanide™ ISA 201 VG, dan vaksin dengan Montanide™ Gel 01. Vaksin diberikan secara intramuskular sebanyak dua kali dengan interval 28 hari. Pengamatan klinis, pengukuran berat badan, dan pengambilan sampel darah dilakukan setiap minggu selama sembilan minggu. Jumlah total eritrosit dianalisis menggunakan alat VetScan HM5 hematology analyzer dan data dianalisis menggunakan repeated measures ANOVA. Tidak terdapat perbedaan signifikan jumlah total eritrosit antar kelompok perlakuan ($p > 0,05$). Namun, waktu pengamatan berpengaruh signifikan terhadap jumlah eritrosit ($p < 0,05$), dengan peningkatan sementara pada minggu ke-3 hingga minggu ke-5. Secara umum, seluruh nilai eritrosit masih berada dalam kisaran fisiologis normal. Disimpulkan bahwa vaksin *S. suis* inaktif dengan adjuvan Montanide™ ISA 201 VG maupun Montanide™ Gel 01 tidak memengaruhi jumlah total eritrosit secara signifikan dan dapat ditoleransi dengan baik oleh anak babi Landrace pada kondisi penelitian ini.

Kata kunci: *Streptococcus suis*, eritrosit, Montanide™, anak babi, keamanan vaksin

INTRODUCTION

Streptococcus suis is one of the most important zoonotic bacterial pathogens in pigs, causing substantial economic losses and public health concerns in many countries, including Indonesia (Liu *et al.*, 2025). Infection with this bacterium can lead to meningitis, septicemia, arthritis, respiratory disorders, and sudden death in pigs (Benea *et al.*, 2025). In addition to its impact on animal health, *S. suis* also poses a significant zoonotic risk, particularly among individuals who have close contact with pigs or pork products (Gajdács *et al.*, 2020). In Bali, this bacterium was reported as a major cause of bacterial meningitis in humans during the period of 2014–2017 (Susilawathi *et al.*, 2019; Tarini *et al.*, 2022). Although *S. suis* has been isolated from clinically affected pigs in Bali (Besung *et al.*, 2019), published surveillance data describing its prevalence, distribution, and epidemiological trends in the province since 2018 remain limited. This knowledge gap highlights the need for continuous epidemiological surveillance and further research to generate evidence for effective prevention and control strategies.

Vaccination is considered one of the most promising approaches for controlling streptococcosis in pigs (Obradovic *et al.*, 2021). Various types of *S. suis* vaccines have been developed, including inactivated whole-cell vaccines, subunit vaccines, and recombinant vaccines (Osterloh, 2022). However, the high diversity of serotypes and antigenic variation among *S. suis* strains remains a major challenge in developing effective vaccines (Fang & Ning, 2025; Segura, 2020). Therefore, the use of local strains as antigen sources is considered advantageous because of their better antigenic compatibility with circulating field isolates. Previous studies have demonstrated that vaccines prepared from local *S. suis* strains are capable of inducing strong antibody responses in experimental mice and pigs (Darawinata *et al.*, 2025; Kaler *et al.*, 2025; Sari *et al.*, 2026). Although the immunogenicity of local *S. suis* vaccines has been well documented, the overall performance of a vaccine also depends on the safety and compatibility of its formulation, including the adjuvant used (Segura, 2020).

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Adjuvants play an important role in vaccine formulations by enhancing immune responses. In the present study, two Montanide™ adjuvants, namely Montanide™ ISA 201 VG and Montanide™ Gel 01, were used. Montanide™ ISA 201 VG is a water-in-oil-in-water emulsion-based adjuvant known to stimulate strong humoral and cellular immune responses, whereas Montanide™ Gel 01 is a synthetic polymer-based adjuvant that generally induces milder immune stimulation (Facciolà *et al.*, 2022; Zhao *et al.*, 2023). Previous studies have reported that both adjuvants can enhance antibody responses against *S. suis* vaccines in experimental pigs (Darawinata *et al.*, 2025; Pratama *et al.*, 2025). Nevertheless, information regarding the effects of these adjuvants on physiological conditions in pigs, particularly total erythrocyte counts, remains limited.

Total erythrocyte count is one of the fundamental hematological parameters used to assess the physiological status and general health of pigs. In healthy piglets, erythrocyte counts generally range from approximately 5.0–8.0 × 10^6/µL, although reference intervals vary according to age, breed, and physiological status. Alterations in erythrocyte counts may reflect changes in erythropoiesis, erythrocyte destruction, blood loss, or systemic disease. Consequently, monitoring erythrocyte counts following vaccination provides useful information for detecting potential adverse physiological effects associated with vaccine formulations or adjuvants (Brooks *et al.*, 2022).

Despite encouraging evidence of vaccine immunogenicity and the ability of Montanide™ adjuvants to enhance antibody responses, information regarding the hematological safety of different adjuvant formulations, particularly their effects on total erythrocyte counts, remains limited. This lack of hematological safety data may hinder the broader application of locally developed *S. suis* vaccines because their physiological safety cannot be comprehensively assessed. Therefore, determining whether different Montanide™ adjuvant formulations influence total erythrocyte counts is essential for supporting their safe application. Accordingly, this study was conducted to evaluate the effects of Montanide™ ISA 201 VG and Montanide™ Gel 01 adjuvants incorporated into a local *S. suis* strain vaccine on total erythrocyte counts in Landrace piglets. The findings of this study are expected to provide baseline information on the hematological safety of these adjuvants and support the development of safe and effective *S. suis* vaccines.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Research Ethics Commission of the Faculty of Veterinary Medicine, Udayana University, Indonesia, under approval number B/181/UN14.2.9/PT.01.04/2024. Prior to the study, the farm owner was informed about the objectives and experimental procedures, and consent was obtained for the use of the piglets in this research.

Experimental Piglets and Husbandry

A total of 12 eight-week-old male Landrace piglets were used in this study. The sample size was determined based on previous experimental studies evaluating the safety and immunogenicity of *S. suis* vaccines in piglets using similar experimental designs (Darawinata *et al.*, 2025; Pratama *et al.*, 2025). The piglets were raised on a pig farm located in Bangbang Village, Bangli Regency, Bali, Indonesia. At the beginning of the experiment, the piglets had a mean body weight of 18.58 ± 1.44 kg. All piglets were clinically healthy, had no history of disease, and had not received any vaccination or medication prior to the experiment. Piglets that developed clinical illness unrelated to vaccination, required therapeutic treatment, or died

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during the experimental period were predetermined as exclusion criteria. However, no animals met these criteria. The piglets were housed in an open-sided concrete pen with a tiled roof measuring 6 × 12 m and maintained under good ventilation conditions. Environmental temperatures ranged from 21–28°C, with relative humidity between 75% and 98%. Piglets were fed a commercial complete prestarter pig feed (BABI A; PT Japfa Comfeed Indonesia Tbk) twice daily. According to the manufacturer's specifications, the diet contained 20% crude protein and approximately 200 mg Fe/kg feed. Drinking water was provided *ad libitum*. Before the experiment began, all piglets underwent a 14-day acclimatization period following previously reported husbandry procedures (Maxwell *et al.*, 2024).

Study Design

This study employed a completely randomized design with repeated measurements. Following the acclimatization period, the piglets were randomly assigned into three treatment groups, each consisting of four piglets: P1, control group receiving sterile 0.9% physiological saline; P2, vaccinated group; and P3, vaccinated group. Piglets in group P2 received the inactivated *Streptococcus suis* vaccine formulated with Montanide™ ISA 201 VG, whereas piglets in group P3 received an inactivated *S. suis* vaccine formulated with Montanide™ Gel 01. Each piglet received 4 mL of the assigned vaccine formulation by intramuscular injection, while piglets in the control group received 4 mL of sterile 0.9% physiological saline. Vaccination was administered twice, consisting of a primary vaccination at week 1 and a booster vaccination at week 5, with a 28-day interval between administrations (Obradovic *et al.*, 2021; Rumapea *et al.*, 2022). All piglets were monitored for nine weeks following the initial vaccination. Clinical monitoring was performed weekly and included rectal temperature measurement, body weight recording, and behavioral observations. Blood samples were collected weekly via the jugular vein. Approximately 3 mL of whole blood was placed directly into EDTA tubes for hematological analysis. A total of 108 blood samples were obtained during the study period.

***Streptococcus suis* Culture**

The *S. suis* IIA3 strain used in this study was obtained from the Biomedical Laboratory culture collection, Faculty of Veterinary Medicine, Udayana University, and had been previously confirmed by polymerase chain reaction (PCR). The isolate was cultured on sheep blood agar (SBA; Difco™, USA) and incubated overnight at 37°C. Five bacterial colonies were subsequently inoculated into 500 mL of tryptone soy broth (TSB; Difco™, USA) and incubated at 37°C for 48 h under continuous agitation. The bacterial suspension was adjusted to a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL, before the inactivation procedure (Kaler *et al.*, 2025). Bacterial purity and morphology were confirmed by subculture on SBA and Gram staining according to the method described by Besung *et al.* (2019).

Bacterial Inactivation

This inactivation approach is consistent with previously reported methods for preparing inactivated *S. suis* vaccines while preserving their immunogenic potential (Obradovic *et al.*, 2021). The bacterial suspension was divided into two 50 mL aliquots and centrifuged at 5000 rpm for 10 min. The supernatant was discarded, and the bacterial pellets were resuspended in sterile 0.9% physiological saline to a final volume of 50 mL. The inactivation process was carried out in two stages. The first stage involved ultrasonication at 70% amplitude for 20 min in three cycles using an ultrasonic processor (Newton, CT, USA). The second stage consisted of heat treatment at 80°C for 2 h using a water bath incubator. Successful bacterial inactivation was confirmed by inoculating the suspension onto Mueller Hinton agar (MHA; Difco™, USA)

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followed by incubation at 37°C for 18 h to ensure the absence of bacterial growth (Besung *et al.*, 2019).

Vaccine Formulation

The inactivated bacterial antigen was formulated into two vaccine candidates using oil-based Montanide™ adjuvants obtained from SEPPIC (Fairfield, NJ, USA). The Montanide™ Gel 01 formulation consisted of 7.5% adjuvant, 42.5% physiological saline, and 50% antigen. Meanwhile, the Montanide™ ISA 201 VG vaccine was prepared by mixing the adjuvant and *S. suis* antigen at a 1:1 volume ratio. To improve emulsion stability, 0.01% v/v polysorbate (Sigma-Aldrich, USA) was added, and the mixture was homogenized using a magnetic stirrer at 1500 rpm for 25 min (Obradovic *et al.*, 2021).

Hematological Analysis

Hematological examination was performed using a VetScan HM5 automated hematology analyzer (Abaxis, Union City, California, USA) on fresh EDTA blood samples. The parameter evaluated in this study was total erythrocyte count. Prior to sample analysis, the instrument was calibrated and quality control procedures were performed according to the manufacturer's instructions. Although the analyzer was capable of measuring additional hematological parameters, including hemoglobin concentration, hematocrit, erythrocyte indices (MCV, MCH, and MCHC), and leukocyte count, the present study focused exclusively on total erythrocyte count because it was the predefined primary outcome for evaluating the hematological safety of the vaccine formulations. All blood samples were analyzed within 2 h of collection to minimize pre-analytical variation and preserve sample integrity.

Statistical Analysis

Data were analyzed descriptively and inferentially using IBM SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA). Differences in erythrocyte counts among treatment groups and observation times were analyzed using repeated measures analysis of variance (repeated measures ANOVA). When significant differences were detected, post hoc analysis was performed using the Least Significant Difference (LSD) test. A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Results

Clinical Observations of Experimental Piglets

Throughout the observation period, all piglets included in this study, including the control group (P1) and the groups vaccinated with inactivated *Streptococcus suis* formulated with Montanide™ ISA 201 VG (P2) or Montanide™ Gel 01 (P3), remained in good health condition. No signs of health disturbances were observed in any of the piglets**.** Clinical manifestations commonly associated with illness, such as high fever, lethargy, or behavioral abnormalities, were not detected in any group during the study period.

Feed intake and activity remained normal throughout the experimental period, and body weight increased progressively in all groups, with no clinically apparent differences among the treatment groups. Rectal temperature remained stable throughout the study, averaging approximately 39.0°C and remaining within the normal physiological range for piglets, including during the periods following the primary (week 1) and booster (week 5) vaccinations. No visible local reactions, such as swelling, redness, or abscess formation at the injection site, were observed following either vaccination. These findings indicate that both vaccine

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formulations containing Montanide™ ISA 201 VG or Montanide™ Gel 01 were well tolerated by the piglets and did not induce clinically detectable local or systemic adverse effects.

Total Erythrocyte Counts

Descriptive data for total erythrocyte counts are presented in Table 1. In general, the control group showed relatively greater fluctuations in erythrocyte counts throughout the observation period, with the highest value reaching 8.38×10^6/µL at week 3 and the lowest value recorded at 5.68×10^6/µL at week 1. The group vaccinated with Montanide™ ISA 201 VG showed a relatively stable pattern, with erythrocyte counts ranging from 5.79×10^6/µL at week 6 to 7.56×10^6/µL at week 3. Meanwhile, the group receiving Montanide™ Gel 01 demonstrated the most stable erythrocyte counts among all groups, with values ranging from 5.37×10^6/µL at week 2 to 6.61×10^6/µL at week 1. The relatively greater fluctuation observed in the control group, particularly the larger standard deviation at several observation points, may reflect normal inter-individual biological variation rather than a treatment-related effect, as the control piglets received only sterile physiological saline.

Statistical analysis using repeated measures ANOVA revealed that observation time significantly affected erythrocyte counts ($p < 0.05$). In other words, the fluctuations in erythrocyte counts observed throughout the study could not be attributed solely to random variation. However, further analysis showed no significant differences among treatment groups ($p > 0.05$). This finding indicates that erythrocyte counts in the control group were not statistically different from those in piglets vaccinated with Montanide™ ISA 201 VG or Montanide™ Gel 01.

To further evaluate differences among observation times, a post hoc Least Significant Difference (LSD) test was performed, and the results are presented in Table 2. Based on Table 2, erythrocyte counts at week 3 differed significantly ($p < 0.05$) only from those observed at weeks 1 and 2, while remaining statistically comparable to the subsequent observation weeks. Visually, the highest erythrocyte values generally occurred between weeks 3 and 5 following vaccination. In contrast, erythrocyte counts at weeks 1, 2, 8, and 9 tended to be lower and relatively homogeneous.

Overall, the main finding of this study was that vaccination with inactivated *S. suis*, either formulated with Montanide™ ISA 201 VG or Montanide™ Gel 01, did not induce notable clinical adverse effects, impair body weight development, or produce significant differences in erythrocyte counts among treatment groups. Nevertheless, observation time influenced erythrocyte fluctuations, with temporary increases occurring between weeks 3 and 5 before gradually declining during subsequent weeks.

Discussion

Safety is one of the primary considerations in vaccine evaluation, particularly to ensure that vaccine formulations do not induce harmful systemic adverse effects in target piglets. In the present study, all piglets receiving the inactivated *Streptococcus suis* vaccine formulated with either Montanide™ ISA 201 VG (P2) or Montanide™ Gel 01 (P3) maintained good clinical condition throughout the nine-week observation period. No clinical signs such as hyperthermia, reduced activity, anorexia, or abnormal behavioral changes were observed.

The body temperature of all piglets remained within the normal physiological range for piglets, averaging approximately 39°C. The absence of significant increases in body temperature suggests that the vaccine formulations did not trigger excessive systemic inflammatory responses. Post-vaccination fever is generally associated with the release of proinflammatory cytokines following stimulation of the innate immune system by antigens and adjuvants

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(Marshall *et al.*, 2018). Therefore, the stable body temperature observed in this study indicates that both adjuvant formulations were capable of stimulating immune responses without inducing excessive systemic inflammation.

The findings of this study are consistent with those reported by Obradovic *et al.* (2021), who demonstrated that *S. suis* bacterin vaccines formulated with various commercial adjuvants showed good tolerability in weaned piglets. Similarly, Facciolà *et al.* (2022) explained that modern emulsion- and polymer-based adjuvants are designed to enhance vaccine immunogenicity while maintaining favorable safety profiles. In another study, Darawinata *et al.* (2025) reported that inactivated *S. suis* vaccines formulated with Montanide™ adjuvants did not induce clinical disturbances or deterioration of general condition in piglets during the observation period. The absence of clinical abnormalities in the present study further suggests that both vaccine formulations possess good biocompatibility in the target piglets. This aspect is particularly important because vaccine safety is a key requirement for the development of practical vaccine candidates suitable for field application in the swine industry.

Body weight development is an important indicator of animal health and welfare during experimental studies. Throughout the observation period, all groups showed gradual and consistent increases in body weight from week 1 to week 9. These findings indicate that the vaccination process did not impair growth performance or adversely affect the physiological condition of the piglets.

No marked differences in body weight gain patterns were observed between the control and vaccinated groups. This finding suggests that administration of the inactivated *S. suis* vaccine formulated with either Montanide™ ISA 201 VG or Montanide™ Gel 01 did not negatively affect metabolism or nutrient utilization efficiency. These results are in agreement with the findings of Obradovic *et al.* (2021), who reported that *S. suis* bacterin vaccines formulated with Montanide™ adjuvants did not interfere with growth performance in piglets. Zhao *et al.* (2023) also noted that modern emulsion-based adjuvants are designed to induce effective immune stimulation with minimal systemic reactions, thereby exerting limited effects on the physiological status of piglets.

The stable growth performance observed in this study also suggests that post-vaccination immune responses did not progress into chronic inflammation. Prolonged systemic inflammation is known to increase protein catabolism and reduce growth efficiency due to the elevated energy demand associated with sustained immune activation (Rauw *et al.*, 2025). However, such conditions were not observed during the study period. Therefore, based on growth performance parameters, both inactivated *S. suis* vaccine formulations can be considered safe and well tolerated under the conditions of this study.

Erythrocytes play a critical role in oxygen transport and in maintaining physiological homeostasis through hemoglobin distribution to body tissues. Therefore, evaluation of erythrocyte counts following vaccination is important to determine whether the vaccine formulations or adjuvants influence erythropoiesis or induce erythrocyte damage such as hemolysis. Descriptively, erythrocyte counts in all groups remained within the normal physiological range throughout the observation period. The control group exhibited relatively greater fluctuations compared with the vaccinated groups, whereas piglets receiving Montanide™ ISA 201 VG or Montanide™ Gel 01 showed more stable erythrocyte patterns. These findings indicate that vaccination did not substantially affect erythrocyte counts.

Repeated measures ANOVA demonstrated that observation time significantly influenced erythrocyte counts ($p < 0.05$), whereas adjuvant type did not significantly affect the parameter ($p > 0.05$). The absence of significant differences among treatment groups suggests that the

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two adjuvants did not induce different levels of hematological stress or disturbances in erythropoiesis. The temporary increase in erythrocyte counts observed between weeks 3 and 5 was more likely associated with physiological growth processes rather than the effects of vaccination itself. Sperling *et al.* (2024) reported that erythrocyte counts in young piglets may fluctuate due to changes in metabolic demand, circulatory system development, nutritional status, and iron balance during active growth phases.

In addition to growth-related factors, mild immune responses following vaccination may also temporarily influence hematological dynamics. Marshall *et al.* (2018) stated that immune activation can induce transient physiological changes in hematological parameters due to the release of low levels of inflammatory mediators. Nevertheless, because these changes did not differ significantly among groups and remained within normal physiological ranges, the fluctuations observed in this study were most likely part of normal biological variation.

Interestingly, although Montanide™ ISA 201 VG is a water-in-oil-in-water emulsion adjuvant known to induce stronger humoral and cellular immune responses (Zhao *et al.*, 2023), this formulation did not produce erythrocyte changes different from those observed with Montanide™ Gel 01, a synthetic polymer-based adjuvant. This finding suggests that both adjuvants demonstrated good compatibility with erythrocyte-related parameters in piglets.

Overall, the results of this study indicate that inactivated *S. suis* vaccines formulated with either Montanide™ ISA 201 VG or Montanide™ Gel 01 did not adversely affect erythrocyte counts in piglets. The stability of erythrocyte counts following vaccination may serve as an important indicator that both vaccine formulations possess good safety characteristics with respect to erythrocyte parameters and may therefore have potential for further development as safe and immunogenic *S. suis* vaccines.

CONCLUSION AND SUGGESTIONS

Conclusion

Inactivated *Streptococcus suis* vaccines formulated with either Montanide™ ISA 201 VG or Montanide™ Gel 01 were well tolerated by Landrace piglets without causing clinical disturbances or impairing growth performance throughout the observation period. In addition, both vaccine formulations did not produce significant differences in total erythrocyte counts among treatment groups. These findings suggest that both adjuvants demonstrated good clinical and erythrocyte-related safety profiles and may have potential for further development as vaccine adjuvants for *S. suis* in piglets.

Suggestions

Further studies are needed to evaluate the protective efficacy of these vaccine formulations against virulent *S. suis* challenge infections. Additional hematological parameters, including differential leukocyte counts, should also be investigated to provide a more comprehensive assessment of vaccine safety. Moreover, comparative studies between Montanide™ adjuvants and other commonly used adjuvants, such as Alum, are recommended.

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Tables

Table 1. Mean Total Erythrocyte Counts (×10⁶/µL) of Piglets in Each Treatment Group Throughout the Observation Period

Group	Week									Overall Mean
	1	2	3	4	5	6	7	8	9	
Control	5.68 ± 0.56	5.74 ± 0.48	8.38 ± 1.31	7.53 ± 1.04	7.46 ± 1.10	7.56 ± 3.04	6.07 ± 0.67	5.86 ± 0.80	5.98 ± 0.73	6.69 ± 1.51[a]
ISA 201 VG	5.90 ± 0.47	6.04 ± 0.49	7.56 ± 1.53	7.19 ± 0.95	6.77 ± 1.06	5.79 ± 0.38	6.84 ± 1.15	6.38 ± 0.71	6.59 ± 0.79	6.56 ± 0.98[a]
Gel 01	6.61 ± 0.39	5.37 ± 0.45	6.39 ± 0.79	6.50 ± 0.50	6.54 ± 0.56	6.29 ± 0.36	6.57 ± 0.95	6.31 ± 0.50	6.32 ± 0.47	6.32 ± 0.63[a]
Overall Mean	6.06 ± 0.60	5.72 ± 0.52	7.45 ± 1.42	7.08 ± 0.90	6.92 ± 0.95	6.57 ± 1.87	6.49 ± 0.92	6.18 ± 0.66	6.30 ± 0.67	6.53 ± 1.11

Note: The same superscript letters indicate no significant differences among groups ($p > 0.05$)

Table 2. Mean Total Erythrocyte Counts (×10⁶/µL) of Piglets at Different Observation Times

Observation Time	Total Erythrocyte Counts (Mean ± SD)
Week 1	6.06 ± 0.60[a]
Week 2	5.72 ± 0.52[a]
Week 3	7.45 ± 1.42[b]
Week 4	7.08 ± 0.90[b]
Week 5	6.92 ± 0.95[b]
Week 6	6.57 ± 1.87[b]
Week 7	6.49 ± 0.92[b]
Week 8	6.18 ± 0.66[ab]
Week 9	6.30 ± 0.67[ab]

Note: Different superscript letters indicate significant differences among observation times ($p < 0.05$).

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