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# Effect of a local foot-and-mouth disease vaccine candidate on TNF-α concentration and muscle necrosis in mice

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**ABSTRACT:** Foot-and-mouth disease (FMD) is a contagious transboundary disease that causes livestock losses. Vaccination is central to FMD control, but its effectiveness depends on the antigenic relevance to field strains. This study evaluated the safety and immunological response of a local isolate-based inactivated FMD vaccine candidate in mice. Eighty BALB/c mice were divided into four groups: adjuvant-only control, inactivated FMD virus antigen at $10^8$ $TCID_{50}$, $10^7$ $TCID_{50}$ with Montanide ISA, and $10^6$ $TCID_{50}$ with Montanide ISA. Immunization occurred intramuscularly on days 0 and 14. Tumor necrosis factor-alpha (TNF-α) concentrations were measured by ELISA, and injection-site tissues were examined histopathologically for necrosis. TNF-α concentrations increased after immunization, peaking post-booster in the adjuvanted $10^7$ $TCID_{50}$ group. Histopathology showed preserved architecture with no detectable necrosis. These findings suggest a systemic immune response without local muscle damage. Further studies in livestock are required to confirm protective efficacy, neutralizing antibody responses, and field-level safety.

**Keywords:**
foot-and-mouth disease, local isolate vaccine, TNF-α, Montanide ISA adjuvant, muscle necrosis.

## ■ INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious transboundary disease of cloven-hoofed livestock that causes major economic losses through reduced productivity, movement restrictions, and trade disruption (Knight-Jones & Rushton, 2013). Vaccination remains the cornerstone of FMD control; however, protection depends on antigenic matching between circulating field viruses and selected vaccine strains (Paton *et al.*, 2005). Because FMDV shows substantial antigenic diversity across serotypes and lineages, local isolate-based vaccine candidates may be more relevant than heterologous imported strains when they reflect currently circulating viruses (Mahapatra & Parida, 2018). However, oil-adjuvanted FMD vaccines may induce persistent local reactions, and excessive TNF-α-mediated signaling can amplify tissue injury and necrosis (Ko *et al.*, 2018; Chen *et al.*, 2018). Therefore, this study evaluated the safety and early immunogenic response of a locally developed FMD vaccine candidate in mice.

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## ■ MATERIALS AND METHODS

This study was approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Airlangga University, Indonesia (approval no. 2.KEH.101.06.2023). Eighty male BALB/c mice aged 6–8 weeks and weighing 20–30 g were assigned to four groups (n = 20/group): K1, adjuvant only; K2, inactivated FMDV antigen at $10^8$ $TCID_{50}$; K3, inactivated FMDV antigen at $10^7$ $TCID_{50}$ with adjuvant; and K4, inactivated FMDV antigen at $10^6$ $TCID_{50}$ with adjuvant. No untreated negative control was included. Immunization was administered intramuscularly (0.1 mL) on days 0 and 14. Animals were observed and sampled on days 0, 14, 21, 28, and 56. Blood was collected for TNF-α quantification by ELISA, and the injection-site muscle was harvested for histopathology. Sections were stained with hematoxylin and eosin and examined at 400× microscopy. Muscle necrosis was scored 0–3: 0, none; 1, <30%; 2, 31–50%; 3, >51%. TNF-α concentrations were analyzed by one-way analysis of variance with Duncan's test; necrosis scores by Kruskal–Wallis and pairwise Mann–Whitney U tests. Statistical significance was set at $P < 0.05$.

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Table 1. TNF-α concentration in mice post-immunization with the candidate FMD vaccine

| Day | Group | | | |
|---|---|---|---|---|
| | K1 | K2 | K3 | K4 |
| 0 | 45.20±24.81$^{ab}$ | 116.43±91.49$^{cd}$ | 94.35±11.94$^{bc}$ | 5.82±1.12$^{a}$ |
| 14 | 89.79±47.76$^{bc}$ | 204.62±17.01$^{ef}$ | 204.16±7.96$^{ef}$ | 7.92±0.68$^{a}$ |
| 21 | 95.52±70.00$^{bc}$ | 199.93±17.53$^{ef}$ | 234.75±30.22$^{fg}$ | 13.31±7.23$^{a}$ |
| 28 | 156.16±79.30$^{de}$ | 274.93±79.74$^{g}$ | 286.25±3.06$^{g}$ | 67.03±14.81$^{bc}$ |
| 56 | 89.03±57.89$^{bc}$ | 179.37±35.99$^{e}$ | 239.47±3.43$^{fg}$ | 10.00±3.27$^{a}$ |

Description: Different superscript letters (a,b,c,d) indicate a significant difference (P<0.05) in TNF-α concentrations between treatment groups and observation days based on the Mann-Whitney test. Groups sharing the same letter are not significantly different (P>0.05).

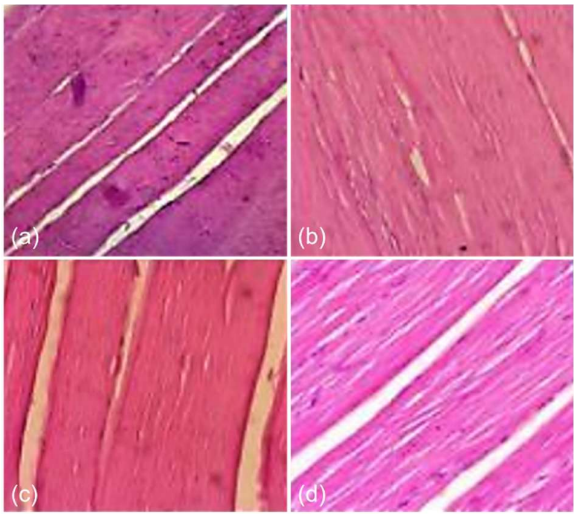


Figure 1. Representative histopathological sections of the mouse thigh muscle at the injection site across different treatment groups and observation periods: (a) K1 on Day 56; (b) K2 on Day 28; (c) K3 on Day 14; and (d) K4 on Day 21. All sections demonstrate healthy muscle architecture with no evidence of necrosis or structural damage HE staining; 400x magnification.

## ■ RESULTS AND DISCUSSION

Inactivated FMD vaccine candidates induced systemic inflammation, as shown by serum TNF-α concentrations (Table 1). TNF-α levels increased post-immunization, peaking on day 28 in groups K1, K2, and K4 ($P < 0.05$), indicating antigen exposure and adjuvant administration activated innate immunity. This pattern aligns with booster vaccination, where repeated antigen exposure enhances immune activation and cytokine release during the transition to adaptive immunity (Cui *et al.*, 2019). Group K3, which was administered $10^7$ $TCID_{50}$ antigen with an adjuvant, showed the highest TNF-α concentration (286.25 pg/mL), and levels declined by day 56 toward homeostasis. Histopathological findings supported safety. From day 0 to 56, hematoxylin and eosin-stained injection-site muscle sections showed preserved architecture without necrosis (Figure 1). Semiquantitative scoring confirmed a median necrosis score of 0 in all groups. Thus, the TNF-α increase, highest in K3, lacked local myotoxicity or injection-site necrosis.

This dissociation between systemic cytokine activation and tissue integrity is critical for vaccine evaluation. Oil-adjuvanted FMD vaccines improve responses; however, safety depends on the adjuvant composition, antigen formulation, route of administration, and injection-site reaction (Choi *et al.*, 2020). Previous studies have reported FMD vaccination-associated lesions and tissue damage at the injection site (Ko *et al.*, 2018). Therefore, the absence of muscle necrosis suggests local tolerance despite inflammatory activation.

These findings support an early safety–immunogenicity balance for vaccine development. The TNF-α response indicates that the formulations were immunologically active, whereas the absence of histologically detectable muscle necrosis suggests no local injury. However, studies in susceptible target animals are required to confirm antibody responses, neutralizing activity, immunity duration, clinical safety, and injection-site tolerability.

## ■ CONCLUSION

After booster immunization, the isolate-based inactivated FMD vaccine candidate induced systemic TNF-α, indicating immune activation, without detectable injection-site muscle necrosis, supporting livestock safety evaluation.

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