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Comparative Analysis of Antibodies Responses Induced by Three Commercial Foot-and-Mouth Disease Vaccines Against Three Virus Serotypes in Egyptian Cattle

Ahmed Mohsen Tahoun[1], Ahmed Elsify[1]*, Mohamed Nayel[1], Ahmed Zaghawa[1], Nourhan Eissa[2], and Omnia H. Refaei[3]

1 *Department of Animal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, University of Sadat City, Sadat City 32897, Menoufia, Egypt.*

2 *Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, University of Sadat City, Sadat City 32897, Menoufia, Egypt.*

3 *Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.*

*Corresponding author: ahmedelsify@gmail.com *Received:* 12/1/2025 *Accepted:* 9/2/2025

ABSTRACT

Vaccination remains the main control practice for foot-and-mouth disease (FMD), particularly in endemic regions like Egypt, where three FMD virus (FMDV) serotypes (A, O, and SAT2) circulate. This study aimed to compare the immunogenicity of three commonly used FMD vaccines in Egypt by evaluating neutralizing antibody responses against three FMDV serotypes (A Iran 05, O Pan-Asia2, and SAT2 ERI) in beef cattle. Thirty-five unvaccinated calves were grouped and vaccinated with either locally produced vaccine A (oil-adjuvanted multivalent), locally produced vaccine B (oil-adjuvanted heptavalent), or imported vaccine C (aluminum hydroxide and saponin-adjuvanted hexavalent). Serum samples were collected at 0, 21, 56, 91, and 126 days post-vaccination (DPV) and tested using the serum neutralization test to determine serotype-specific $\log_{10}$ antibody titres. Baseline antibody levels at 0 DPV were below the protective threshold ($\log_{10} \geq 1.5$) for all groups. Vaccine A induced significantly lower antibody titres compared to vaccines B and C, with no significant changes in antibody titres across all time points, raising concerns about its efficacy. Vaccine B elicited sustained protective titres against all serotypes until 126 DPV. Vaccine C showed an initial protective response, but titres waned significantly below the protective threshold by 91 DPV. Additionally, Protective titre against SAT2 ERI was achieved following a booster dose of vaccine C. These findings highlight the superior performance of vaccine B in inducing durable and rapid protective immune responses, likely due to its oil-based adjuvant system. Both vaccines B and C are recommended for inclusion in vaccination campaigns, with selection depending on financial considerations since vaccine C requires revaccination within shorter intervals.

Keywords: Foot-and-mouth disease, Vaccine, Antibody response, Post-vaccination, Serum neutralization test.

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral disease caused by the foot-and-mouth disease virus (FMDV), an icosahedral, non-enveloped virus belongs to Aphthovirus genus in the Picornaviridae family (Tekleghiorghis et al., 2016). The virus genome is a single-stranded RNA capable of initiating replication independently of viral proteins (Diab et al., 2015). The virus exhibits significant genetic and antigenic variability, leading to the identification of seven immunologically distinct FMDV serotypes: A, O, C, Southern African Territories 1 (SAT1), SAT2, SAT3, and Asia 1, each comprising multiple subtypes. Viral quasispecies generated during replication are undergo selection based on environmental conditions, fitness, and immune pressures (Arzt et al., 2019; Tulloch et al., 2018).

Cloven-hoofed domestic animals, including cattle, sheep, goats, and pigs, and over 70 species of wild animals are susceptible to FMDV infection resulting in substantial economic losses, particularly in dairy herds, due to decreased productivity, trade restrictions, and the costs associated with control measures (Knight-Jones et al., 2017; Paton et al., 2018; Rushton and Knight-Jones, 2015). Cattle with FMD show severe clinical symptoms, including fever, reduced milk production, and vesicular lesions on the oral mucosa, feet, teats, and other skin areas. Young calves often exhibit high mortality rates due to myocarditis (Alexandersen and Mowat, 2005; Azeem et al., 2020). In certain instances, FMD outbreaks can result in high morbidity and mortality among milking cows. This is often associated with an increased viral load, frequent exposure, and poor matching between the vaccine strain and the field virus strain (Refaei et al., 2020).

In Egypt, FMD has been endemic for decades since its first documentation in the 1950s (Hefnawy et al., 2018). Three serotypes have been identified: serotype A, which includes the Africa, Asia, and Europe–South America (EURO-SA) topotypes; serotype O, which includes the Middle East-South Asian (ME-SA), East Africa 3 (EA-3), and EURO-SA topotypes; and serotype SAT2 which includes the SAT2 VII topotype (Hagag et al., 2023; Hassanein et al., 2024; Soltan et al., 2022).

Vaccination remains the primary approach for controlling FMD in endemic regions. In Egypt, The national control strategy focuses on mass vaccination campaigns targeting cattle, buffalo, sheep, and goats using both locally produced and imported inactivated vaccines (Al-Hosary et al., 2019). However, variations in vaccine formulations, including the number of strains included, antigen load, and adjuvant types, may influence the immunogenicity and protective efficacy of these vaccines (Bazid et al., 2023). Therefore, evaluating the antibody responses elicited by different FMD vaccines is crucial for optimizing vaccination strategies Moreover, post-vaccination assessment of immune responses is essential to ensure effective vaccine application and to evaluate the herd-level protective immunity. This, in turn, aids in refining the implementation of the control program (Singh et al., 2019). The neutralization test, used for measuring FMDV-specific neutralizing antibodies, is a precise method for assessing serotype-specific protective antibody titers induced by vaccination (Sala et al., 2023).

This study aimed to compare the neutralizing antibody levels elicited by three commonly used FMD vaccines in Egypt against three FMDV serotypes (A Iran 05, O Pan-Asia2, and SAT2 ERI). The evaluated vaccines included two locally produced oil-adjuvanted multivalent vaccines and one imported aluminium hydroxide and saponin-adjuvanted vaccine. Antibody response dynamics were assessed at multiple time points post-

vaccination in beef cattle, and statistical methods were employed to analyse differences in vaccine performance. This study provides essential insights into the relative efficacy of these vaccines, supporting decision-making for vaccination policies in Egypt.

MATERIALS AND METHODS

1. Foot and mouth disease vaccines

Three inactivated FMD vaccines, commonly used for FMD vaccination in Egypt, were utilized in this study: i) Vaccine A: a multivalent, locally produced vaccine containing the following FMDV strains: A Iran 05, A Africa GIV 2020, A Africa GIV 2022, A Euro South America (A Venezuela), O Pan Asia 2, SAT2 Libya 2012, and SAT2 Egypt 2018. The vaccine is formulated with an oil adjuvant, ii) Vaccine B: a heptavalent, locally produced vaccine containing ≥ 6 protective dose 50 (PD_{50}) for each of the following virus strains: A Africa GIV, A Iran 05, O Pan-Asia2, O EA.3, O Manisa 69, SAT2 ERI/98, and SAT2 LIB-12. This vaccine also uses an oil adjuvant, and iii) Vaccine C: an imported hexavalent vaccine containing ≥ 6 PD_{50} for each of the following virus strains: A Iran 05, A Saudi 95, O Manisa, O-3039, SAT2 Eritrea, and Shamir strain of Asia1. This vaccine uses aluminium hydroxide and saponin as adjuvants.

The first two vaccines (vaccines A and B) are included in the mandatory vaccination campaigns implemented by the local governorate authorities.

2. Study population and animal sampling

The study was conducted from October 2022 to February 2023 on two Egyptian beef cattle farms randomly selected from different governorates (selected animals were examined in the same time, same breed and management system but the animals number in one farm not sufficient to do whole experiment in one farm). Farm I is located in Menoufia governorate, while Farm II is located in Sharkia governorate. A total of 35 newly purchased calves, with no history of FMD previous vaccination, were included in the study. All calves were of mixed breed, with an average body weight of 150 kg at the start of the study. The average body weight of animals at the end of the fattening period ranges between 400 and 450 kg.

3. Vaccination protocol and serum samples

The calves were grouped and vaccinated as follows:

- Group A (n = 10): Calves from Farm I vaccinated with Vaccine A.
- Group B (n = 10): Calves from Farm I vaccinated with Vaccine B.
- Group C (n = 15): Calves from Farm II vaccinated with Vaccine C.

A total of 175 serum samples were collected throughout the study. Serum samples were collected from all enrolled calves at the following time points: 0, 21 (time of booster dose administration), 56-, 91-, and 126-days post-vaccination (DPV). Each vaccine dose (2 mL) was administered subcutaneously after thorough mixing. All separated sera were heat inactivated in water bath for 30 minutes at 56°C for non-specific inhibitors elimination then stored at -20°C until analysed by serum neutralization test (SNT).

4. Serum neutralization test

The antibody titers specific to each FMDV serotype were measured using the SNT. The test was performed in biosafety level 3 laboratories. The SNT was carried out according to **(OIE, 2022)** against FMDV (serotypes A Iran 05, O Pan-Asia2 and SAT2 ERI). Briefly, serum samples were initially diluted in cryotube into 1/4 dilution then 2-fold serially diluted in the microtiter plate started from 1/8 to 1/64 in maintenance media (minimum essential

medium (MEM) with Earl's salts) and tested against pre-titrated 100 $TCID_{50}$ (50% tissue culture infective dose)/ 50µl of each FMDV serotype. Baby Hamster Kidney cells (BHK_{21}) in tissue culture grade flat-bottomed microtiter plates were used. The titres were determined as the reciprocal of the highest serum dilution that neutralizes the virus and were expressed as log_{10} values.

5. Statistical analysis:

The data on the log_{10} antibody titres for the three vaccines at different time points were organized using Microsoft-Excel ® data spreadsheet. All statistical analyses were conducted using JASP, Version 0.19.2 (JASP Team, 2024). To evaluate differences in antibody titres between vaccines and across various DPV for each FMDV serotype, non-parametric tests were employed, as the data were not-normally distributed according to the Shapiro-Wilk test at 0.05 significance level. The Kruskal-Wallis test was used as non-parametric test for unpaired samples to compare significant differences between vaccines at each time point. The Friedman test was applied as a non-parametric test for paired samples to assess significant variations across different DPV for each vaccine separately. Pairwise comparisons of groups of different time points and vaccines were performed using Bonferroni-adjusted post hoc tests to control for Type I error. Statistical significance was set at $p < 0.05$.

RESULTS

At zero DPV, the median antibody titre against the three FMDV serotypes for all vaccinated animal groups was 0.6 log_{10} (Figure 1, Table 1). Kruskal-Wallis test results revealed no significant differences in antibody titres among the three FMD vaccines against A Iran 05 ($P = 0.278$), O Pan-Asia2 ($P = 0.163$), and SAT2 ERI ($P = 0.272$) at 0 DPV (Table 2).

Table 1. Descriptive statistics showing the median antibody titers (log_{10}) and interquartile range (IQR) values for three vaccines (A, B, C) against foot and mouth disease virus serotypes (A Iran 05, O Pan-Asia2, and SAT2 ERI) at various days post-vaccination (DPV). The number of animals per group and the valid samples for the serum neutralization test are presented for each vaccine at each time point. Median values were used to describe the data due to its non-normal distribution.

(DPV)	0			21			56			91			126		
vaccine	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
No. of animals /group	10	10	15	10	10	15	10	10	15	10	10	15	10	10	15
Valid samples	9	9	13	9	8	13	10	10	14	10	9	15	10	10	15
(A Iran 05)															
Median	0.6	0.6	0.6	1.2	1.65	1.5	1.2	1.5	1.8	0.6	1.5	1.2	0.6	1.5	0.9
IQR	0	0	0	0.3	0.3	0.3	0.75	0.3	0.3	0.225	0.3	0.5	0	0	0.6
(O Pan-Asia2)															
Median	0.6	0.6	0.6	0.9	1.5	1.5	0.75	1.8	1.8	0.9	1.5	1.2	0.6	1.5	0.9
IQR	0	0	0.3	0.3	0.15	0	0.825	0.225	0.3	0.825	0.3	0.3	0	0.225	0.3
(SAT2 ERI)															
Median	0.6	0.6	0.6	1.2	1.5	1.2	0.6	1.5	1.5	0.6	1.5	1.2	0.6	1.5	0.9
IQR	0	0	0	0.3	0.3	0.3	0	0	0	0.225	0	0	0	0.225	0.45

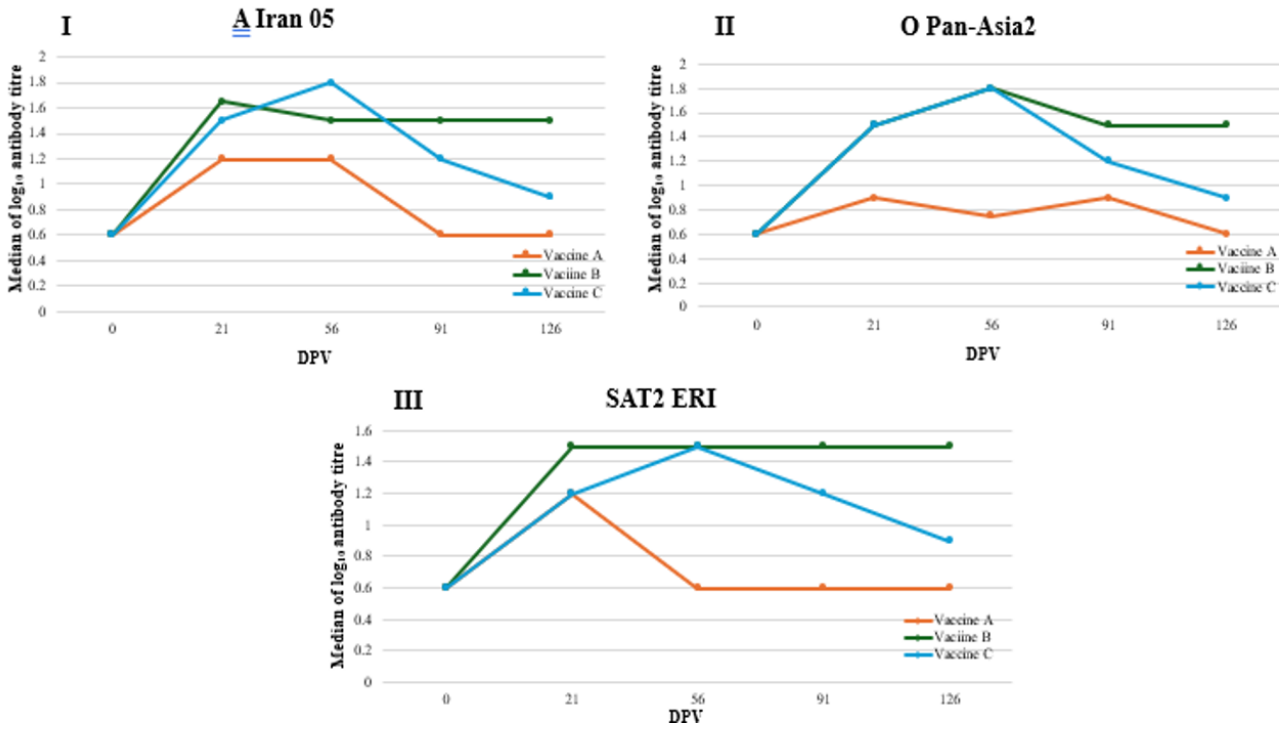

Figure 1. Median values of log_{10} antibody titers using serum neutralization test over days post-vaccination (DPV) for three vaccines (A, B, and C) against different foot-and-mouth disease virus serotypes: (I) A Iran 05, (II) O Pan-Asia2, and (III) SAT2 ERI.

Table 2. Results of Kruskal-Wallis Test for detection the significant changes in antibody titers (log_{10}) among the three foot-and-mouth disease vaccines at different days post-vaccination (DPV), evaluated against three FMD virus serotypes included in each vaccine. The significant *p*-values (< 0.05) are bold.

DPV	Kruskal-Wallis Test (*P* value)		
	A Iran 05	**O Pan-Asia2**	**SAT2 ERI**
0	2.558 (0.278)	3.628 (0.163)	2.601 (0.272)
21	15.356 (**<0.001**)	13.141 (**< 0.001**)	8.978 (**0.011**)
56	10.06 (**0.007**)	13.343 (**0.001**)	21.708 (**<0.001**)
91	13.066 (**0.001**)	5.089 (0.079)	13.491 (**0.001**)
126	14.815 (**<0.001**)	15.019 (**<0.001**)	20.022 (**<0.001**)

For all three FMDV serotypes, the median antibody titres of vaccine A at various time points following 0 DPV were lower than those of vaccine B and C, except for SAT2 ERI at day 21 post-vaccination, where the median of antibody titre (1.2) is equivalent to that of vaccine C (Figure 1, Table1).The median of antibody titres for each FMD vaccine (A, B, and C) across different DPVs against the three FMDV serotypes (A Iran 05, O Pan-Asia2, and SAT2 ERI) are shown in Table 1.

According to Kruskal-Wallis test, significant differences in antibody titres of the three FMDV serotypes were detected

between the three vaccines (p <0.05) at DPV following day 0, except for O Pan-Asia2 at 91 DPV, there is no significant differences (P = 0.079) were observed between three vaccines (Table2).

Comparison of pairwise groups of vaccines along the post-vaccination time points revealed that vaccine A elicited significantly lower antibody response than vaccine B and C (Table 3). No significant differences were found in antibody responses between vaccine B and C for the three FMD serotypes at 21, 56, 91, and 126 DPV, except for A Iran 05 (P_{bonf} = 0.01) and SAT2 ERI (P_{bonf} = 0.032) at 126 DPV (Table 3). The pairwise comparisons of the three vaccines against each FMDV serotype are presented in Table 3.

Table 3. Pairwise comparisons of antibody titers (log_{10}) of three foot and mouth disease vaccines at different days post-vaccination (DPV) against three foot-and-mouth disease virus serotypes. The table presents the Z-scores (Z) and Bonferroni-adjusted p-values ($P_{bonf.}$) for comparisons between vaccine groups A, B, and C at each DPV. Statistically significant differences ($P_{bonf.}$ < 0.05) are highlighted in bold.

DPV	Pairwise groups	A Iran 05		O Pan-Asia2		SAT2 ERI	
		Z	**P_{bonf}**	**Z**	**$P_{bonf.}$**	**Z**	**$P_{bonf.}$**
0	**A vs B**	1.569	0.35	0.669	1	0.645	1
	A vs C	1.115	0.795	-1.133	0.771	-0.89	1
	B vs C	-0.59	1	-1.86	0.189	-1.591	0.335
21	**A vs B**	-3.903	**<0.001**	-2.968	**0.009**	-2.894	**0.011**
	A vs C	-2.355	0.056	-3.334	**0.003**	-2.186	0.086
	B vs C	1.948	0.154	-0.008	1	1.019	0.924
56	**A vs B**	-2.489	**0.038**	-3.236	**0.004**	-3.592	**<0.001**
	A vs C	-2.999	**0.008**	-3.173	**0.005**	-4.437	**<0.001**
	B vs C	-0.311	1	0.323	1	-0.558	1
91	**A vs B**	-3.6	**<0.001**	-2.237	0.076	-3.63	**<0.001**
	A vs C	-2.191	0.085	-0.949	1	-2.407	**0.048**
	B vs C	1.802	0.215	1.519	0.386	1.625	0.312
126	**A vs B**	-3.696	**<0.001**	-3.864	**<0.001**	-4.473	**<0.001**
	A vs C	-1.127	0.779	-1.87	0.184	-2.347	0.057
	B vs C	2.922	**0.01**	2.363	0.054	2.553	**0.032**

The non-parametric Friedman test was applied to each FMD vaccine separately. For vaccine A, the Friedman test indicated no significant differences in antibody titres against the three FMDV serotypes across the five time points, except for SAT2 ERI serotype, a significant decrease in antibody titre against SAT2 ERI was detected between 21 and 126 DPV ($P_{bonf.}$ = 0.021) (Table 4). This can also be observed in figure 1, III.

Table 4. Pairwise comparisons of antibody titers (log_{10}) for vaccine A at different days post-vaccination (DPV) for three foot and mouth disease virus serotypes. The table shows the t-statistics (T-Stat) and Bonferroni-adjusted *p*-values ($P_{bonf.}$) for each comparison, with statistically significant comparisons ($P_{bonf.} < 0.05$) indicated in bold. The Friedman Test results (X^2_F and *P*-values) for each serotype are also included.

Pairwise groups		A Iran 05[a]		O Pan-Asia2[b]		SAT2 ERI[c]	
		T-Stat	$P_{bonf.}$	**T-Stat**	$P_{bonf.}$	**T-Stat**	$P_{bonf.}$
DPV	**0 vs 21**	1.406	1	1.852	0.745	2.778	0.097
	0 vs 56	2.611	0.143	2.161	0.394	0.121	1
	0 vs 91	0.904	1	1.647	1	1.57	1
	0 vs 126	0.402	1	0.515	1	0.604	1
	21 vs 56	1.205	1	0.309	1	2.898	0.072
	21 vs 91	0.502	1	0.206	1	1.208	1
	21 vs 126	1.808	0.814	1.338	1	3.381	**0.021**
	56 vs 91	1.707	0.988	0.515	1	1.691	1
	56 vs 126	3.013	0.054	1.647	1	0.483	1
	91 vs 126	1.306	1	1.132	1	2.174	0.383

[a] Friedman Test (X^2_F) value 9.246, $P = 0.055$
[b] Friedman Test (X^2_F) value 6.33, $P = 0.176$
[c] Friedman Test (X^2_F) value 11.574, $P =$ **0.021**

For each B and C vaccines, significant differences in antibody titres across different DPV (P <0.05) were found for the three FMDV serotype (Table 5 and Table 6). The results of pairwise comparisons for vaccine B and vaccine C against the three FMDV serotypes are detailed in Table 5, and Table 6, respectively.

Table 5. Pairwise comparisons of antibody titers (log_{10}) for vaccine B at different days post-vaccination (DPV) for three foot and mouth disease virus serotypes. The table shows the t-statistics (T-Stat) and Bonferroni-adjusted *p*-values ($P_{bonf.}$) for each comparison, with statistically significant comparisons ($P_{bonf.} < 0.05$) indicated in bold. The Friedman Test results (X^2_F and *P*-values) for each serotype are also included.

Pairwise groups		A Iran 05[a]		O Pan-Asia2[b]		SAT2 ERI[c]	
		T-Stat	$P_{bonf.}$	**T-Stat**	$P_{bonf.}$	**T-Stat**	$P_{bonf.}$
DPV	**0 vs 21**	4.231	**0.004**	5.062	**<0.001**	4.248	**0.004**
	0 vs 56	3.967	**0.008**	4.484	**0.002**	4.248	**0.004**
	0 vs 91	3.306	**0.035**	4.195	**0.004**	3.809	**0.011**
	0 vs 126	3.702	**0.014**	2.893	**0.09**	4.541	**0.002**
	21 vs 56	0.264	1	0.579	1	0	1
	21 vs 91	0.926	1	0.868	1	0.439	1
	21 vs 126	0.529	1	2.17	0.423	0.293	1
	56 vs 91	0.661	1	0.289	1	0.439	1
	56 vs 126	0.264	1	1.591	1	0.293	1
	91 vs 126	0.397	1	1.302	1	0.732	1

[a] Friedman Test (X^2_F) value 13.105, $P =$ **0.011**
[b] Friedman Test (X^2_F) value 14.895, $P =$ **0.005**
[c] Friedman Test (X^2_F) value 14.189, $P =$ **0.007**

Table 6. Pairwise comparisons of antibody titers (log_{10}) for vaccine C at different days post-vaccination (DPV) for three foot and mouth disease virus serotypes. The table shows the t-statistics (T-Stat) and Bonferroni-adjusted *p*-values ($P_{bonf.}$) for each comparison, with statistically significant comparisons ($P_{bonf.} < 0.05$) indicated in bold. The Friedman Test results (X^2_F and *P*-values) for each serotype are also included.

Pairwise groups		A Iran 05[a]		O Pan-Asia2[b]		SAT2 ERI[c]	
		T-Stat	***P*bonf.**	**T-Stat**	***P*bonf.**	**T-Stat**	***P*bonf.**
DPV	**0 vs 21**	8.165	**<0.001**	11.031	**<0.001**	9.431	**<0.001**
	0 vs 56	12.411	**<0.001**	8.12	**<0.001**	13.059	**<0.001**
	0 vs 91	7.512	**<0.001**	7.048	**<0.001**	6.348	**<0.001**
	0 vs 126	2.123	0.4	2.145	0.381	1.088	1
	21 vs 56	4.246	**0.001**	2.911	0.059	3.627	**0.008**
	21 vs 91	0.653	1	3.984	**0.003**	3.083	**0.037**
	21 vs 126	6.042	**<0.001**	8.886	**<0.001**	8.343	**<0.001**
	56 vs 91	4.899	**<0.001**	1.072	1	6.711	**<0.001**
	56 vs 126	10.288	**<0.001**	5.975	**<0.001**	11.97	**<0.001**
	91 vs 126	5.389	**<0.001**	4.903	**<0.001**	5.26	**<0.001**

[a] Friedman Test (X^2_F) value 36.611, $P <$ **0.001**
[b] Friedman Test (X^2_F) value 35.306, $P <$ **0.001**
[c] Friedman Test (X^2_F) value 37.796, $P <$ **0.001**

DISCUSSION

Foot and mouth disease is an endemic viral infection in Egypt. Repeated outbreaks had been recorded in last decade although the use of different commercial inactivated vaccines with observation of emerging of new FMDV strains within the Egyptian field. Which may be related to frequent importation of live animals from different countries (Al-Hosary et al., 2019). Effective control policy should include strict biosecurity measures with periodic mass vaccination campaigns as well as frequent epidemiological monitoring of the disease (Bazid et al., 2023). Vaccination is a valuable tool in the fight against FMD in endemic nations. Unfortunately, current conventional vaccinations give only short-term, serotype-specific protection (Guzman et al., 2010); Furthermore, protection against FMD is mostly based on vaccine efficacy because alternative measures, such as animal movement limitations or biosecurity exercises, are difficult to implement in many countries (Knight-Jones et al., 2016). immunization of young cattle has confusing results since the timing of immunization, maternal antibody level, breed of animal, and adjuvants employed may all influence the antibody response (Hodgins et al., 2004). Successful control programs especially in endemic regions such as Egypt could be achieved by vaccination using high quality prepared inactivated vaccines containing the locally circulated serotypes (Lyons et al., 2016). So that this study aimed to evaluate the immunogenicity of three commonly used FMD vaccines in Egypt by comparing their antibody titers against three FMDV serotypes (A Iran 05, O Pan-Asia2, and SAT2 ERI) at various time points post-vaccination.

The control of the experiment is elucidated in the descriptive statistics includes the median antibody titres (log_{10}) and interquartile range (IQR) values for three vaccines (A, B, C) against foot and mouth disease virus serotypes (A Iran 05, O Pan-Asia2, and SAT2 ERI) at various days post-vaccination (DPV). The median log10 antibody titre (0.6) against all three FMDV serotypes for vaccinated animals at zero DPV was below the protective serum neutralizing antibody threshold (≥ 1.5), as reported by (El-Sayed et al., 2012). No

significant changes in antibody titres were observed among the three vaccine groups at zero DPV, consistent with the animals' vaccination history indicating that they had not previously received FMD vaccines.

The comparative statistical studies between the antibody's immune response through the study period between the three vaccines A, B and C. revealed that the three vaccines revealed that vaccines B and C induced higher antibody titres than vaccine A across the three FMDV serotypes and at various time points post-vaccination. Vaccine A consistently showed significantly lower antibody titres than vaccine B at all time points post-vaccination, except at 91 DPV against the O Pan-Asia2 serotype. Vaccine C elicited significantly higher antibody titres at 56 DPV for all three serotypes, at 21 DPV for O Pan-Asia2, and at 91 DPV for SAT2 ERI than vaccine C. These findings suggest that vaccines B and C provide superior immunogenicity, making them more effective candidates for vaccination campaigns. Vaccines B and C showed no significant differences in antibody titres for all three serotypes at most time points post-vaccination. However, at 126 DPV, vaccine B elicited significantly higher antibody titres than vaccine C for the A Iran 05 and SAT2 ERI serotypes. This indicates that vaccine B induces a more durable and robust neutralizing antibody response. The enhanced performance of vaccine B could be attributed to its oil-based adjuvant system, in contrast to vaccine C, which uses aluminium hydroxide and saponin adjuvants. Oil-based adjuvants are known to elicit longer-lasting antibody responses (Bazid et al., 2023).The level of antibodies post vaccination throughout the period of study agreed with Doel 2003, Massicame 2012, and Cox et al., 2003. They reported that; peak antibody titres were obtained 21 and 35 days after the initial vaccination. These investigations demonstrated that inoculated calves responded quickly to the initial dosage, with peak antibody titres often occurring 14 to 28 days after vaccination. In sheep, the immunological response after a first dosage resulted in antibody generation as early as 7 days post-vaccination, with most animals attaining maximal antibody titres within 28 days. As well as The majority of the elicited immune responses fell between 1.5 and 2 log10 titres. These transient antibodies appear to have mostly decoupled after 4 months of immunization, indicating that naive cattle may require more than one initial vaccine. Following a single vaccination this observation agreed with (Tegegne et al., 2024).

Our findings were also consistent with those of El-Bagoury et al., 2013, who discovered that protective neutralizing serum antibody titers began within the first month of vaccination, with a mean serum-neutralizing antibody titer of 1.7 $\log_{10}$ for serotype "A/Egypt/2006" and 1.6 $\log_{10}$ for serotype "O1/3/93" FMD virus, respectively. According to the findings of the current study, a single dosage of each monovalent vaccine may protect at least 50% of the animals against FMD for a limited time, but a booster immunization may be required to provide protection for a longer term. This is further corroborated by predicted probability logistic regression, as a 120-antibody titer at day 56 post-vaccination was shown to protect 50% of the animals immunized (Barnett et al. 2003).

In the present study vaccine A showed no significant changes in antibody titres against the A Iran 05 and O Pan-Asia2 serotypes throughout the study period (0–126 DPV), indicating a minimal capacity to elicit an effective antibody response. Although a significant change was observed in the antibody titres against the SAT2 ERI serotype, this change resulted from a significant decrease in antibody levels between 21 and 126 DPV. This similarly observed by Patil et al., 2002, Manzoor et al., 2015 and Tegegne et al., 2024. These findings suggest that the potency of vaccine A is questionable from

the aspect of serum neutralizing antibody production. Or may be related to the differences in vaccination top-types (Doel et al., 2033 and Tegegne et al., 2024).

The results of this work showed that vaccine B demonstrated a robust antibody response, maintaining a high protective titter (≥ 1.5) against all three serotypes throughout the study period, up to 126 DPV. A significant increase in antibody titres was observed at 21 DPV, coinciding with the booster dose of the vaccine. Furthermore, the significant difference in titres between zero and 126 DPV highlights that vaccine B sustained protective antibody levels without the need for revaccination during this period. A recently conducted study on vaccine B reported similar antibody titres and persistence against the three serotypes, supporting the suitability of inactivated vaccines with higher potency and effectiveness for controlling FMD in endemic regions (Bazid et al., 2023).

Vaccine C also induced a significant increase in antibody titres for all serotypes at 21 DPV, demonstrating its efficacy in enhancing the immune response. However, unlike vaccine B, the antibody titres for vaccine C waned significantly after 56 DPV. By 91 DPV, the titres had dropped to 1.2, below the protective threshold (≥ 1.5), indicating the necessity for revaccination before 91 DPV. Moreover, regarding the SAT2 ERI serotype, vaccine B achieved protective titres by 21 DPV before the vaccine booster dose, whereas vaccine C required a booster dose to achieve protective levels at 56 DPV. This is consistent with the findings of (Brun et al. 1976; Doel, 1996; Parida, 2009; OIE, 2012), they found that protective antibody levels created by a single vaccine are typically short-lived, lasting only a few months and necessitating regular revaccination for prophylactic control. As well as agreed with Doel, 2003; demonstrated that boosting the immune response by repeated vaccination significantly increases both the magnitude and duration of neutralizing antibody responses.

The dynamic of antibody responses of vaccine B and C over time also highlights the crucial role of adjuvants in influencing the rapidity and durability of humoral immune responses. Vaccine B's oil-based adjuvant system appears to enhance the longevity and maintenance of antibody levels, and this agreed with Garçon et al., 2011 who reported that the oil adjuvant enhancing the immune response; while the aluminium hydroxide and saponin-based adjuvants in vaccine C may contribute to the observed decline in antibody titres over time. This is consistent with Cloete et al., 2008 and Patil et al., 2012, who described the weak immune response induced by inactivated FMD vaccine made with aluminum hydroxide gel and saponin, requiring relatively frequent re-vaccinations to provide protective immunity.

These findings provide valuable insights into the differential performance of FMD vaccines used in Egypt. However, the study focused exclusively on antibody responses, which represent only one aspect of immunity. Future research incorporating assessments of cellular immune responses and matching test measuring r1 values between vaccinal and field strains, should be conducted to offer a more comprehensive evaluation of vaccine effectiveness.

CONCLUSION

Both locally produced vaccine B and imported vaccine C demonstrated better immunogenicity against FMDV 05, O Pan-Asia2, and SAT2 ERI serotypes in Egypt than locally produced vaccine A. vaccine B offers superior antibody response in terms of longevity and maintenance of protective level, Moreover, it induces protective titre more quickly than vaccine C for the SAT2 ERI serotype. These differences could be attributed to variations in the adjuvant formulations of the vaccines, further emphasizing the role

of adjuvants in shaping immune responses. Accordingly, we recommended both vaccines B and C as potentially effective vaccines for controlling FMD in Egypt. However, vaccine C required revaccination within shorter period (about 90 days following primary vaccination) compared to vaccine B, which could be problematic due to financial constraints.

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