VacciMonitor 2025;34:e163425
www.vaccimonitor.finlay.edu.cu
Original Article

Monitoring foot and mouth disease vaccination efficacy based on experimental and field comparisons: from evaluation to protection

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Foot-and-mouth disease remains endemic in Egypt due to the co-circulation of multiple serotypes. Vaccination is the cornerstone of control efforts; however, the emergence of new strains requires regular assessment of vaccine efficacy. This study evaluated a locally produced polyvalent inactivated foot-and-mouth disease vaccine targeting six prevalent serotypes under both experimental and field conditions. Forty-two seronegative calves were experimentally vaccinated and later challenged with homologous strains to assess protection. Simultaneously, a field evaluation was conducted in 600 cattle distributed across 20 Egyptian governorates. Immune responses were measured using virus neutralization tests and AsurDx FMD Multispecies Antibodies ELISA test kit, while vaccine purity was confirmed by detecting non-structural protein antibodies. All experimentally vaccinated animals developed neutralizing antibody titers above the protective threshold ($\geq$1.65 $\log_{10}$ $TCID_{50}$), achieving 100 % protection against four strains. In the field, vaccinated cattle exhibited sustained protective titers for up to 4 months post-vaccination, although a decline in titers was observed against the SAT2 GH strain. Non-structural protein testing confirmed vaccine purity, with more than 80 % of animals testing negative across all surveyed governorates. Mixed-effects regression analysis showed a strong positive correlation between experimental and field virus neutralization test titers (β = 0.71, $p < 0.001$), supporting the extrapolation of laboratory findings to field conditions. Overall, the vaccine was demonstrated to be safe, immunogenic, and broadly effective under diverse conditions. Continued monitoring of circulating strains and timely vaccine updates are essential to sustain effective foot-and-mouth disease control in endemic regions like Egypt.

Keywords: foot-and-mouth disease; vaccine potency; vaccination coverage; serologic tests; comparative study.

Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting cloven-hoofed animals, causing severe economic losses in agriculture and livestock production.(1) This disease affects various livestock, including cattle, swine, sheep, and goats.(2) It is characterized by vesicles in the mouth, tongue, hooves, and nipples, along with an increase in body temperature and appetite loss.(3) Foot-and-mouth disease virus (FMDV) is a small, non-enveloped, single-stranded RNA virus. It possesses a positive-sense RNA genome of approximately 8,500 bases and exhibits both antigenic and genotypic distinctions, allowing its classification into seven immunologically distinct serotypes: O, A, C, Asia 1, South African Territories (SAT) 1, SAT 2, and SAT 3, which belong to the genus

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Aphthovirus, family *Picornaviridae*.[4] Several of these serotypes circulate currently or periodically in the Middle East and North Africa. Since these seven serotypes do not provide cross-protection, frequent updates in vaccination strategies are necessary.[5] FMD presents a global challenge, with each serotype requiring specific vaccines to ensure effective control and prevention.

FMDV spreads through direct or indirect contact with infected animals, their secretions, or contaminated feed. Additionally, airborne transmission can occur over long distances via infectious aerosols (droplets).[6] The risk of virus introduction is further heightened by factors such as the movement of animals and animal products, human mobility, and interactions between domestic and wildlife populations.[7] The insidious nature of the virus allows its detection in milk and semen before clinical signs such as fever and blistering of the oral cavity, teats, and interdigital spaces appear.[8] While often nonfatal in mature animals, FMD poses a severe threat to young animals, leading to myocarditis and substantial production losses. Recovered animals can become intermittent carriers, potentially triggering future outbreaks.[9]

FMD has been endemic in Egypt since the 1950s, with multiple serotypes circulating and causing significant outbreaks over the decades. The first incursion was recorded with serotype O, which established a lasting presence and has remained the dominant strain, causing regular outbreaks. Egypt reported its first case of serotype A in the 1960s, with subsequent outbreaks in 1967 and 1972.[10] In 2006, a new East African type A strain was introduced, revealing genetic similarities to strains found in East Africa. Between 2010 and 2015, another strain of serotype A, identified as A-Iran05-08, was detected in Egypt, indicating its introduction from Iran.[9]

A significant shift occurred in 2012 when the first recorded outbreak of serotype SAT2 (topotype VII) led to six reported outbreaks across multiple Egyptian governorates. The African type-G-IV variant of SAT2 was later detected in 2012 and continued to be reported in subsequent years, including outbreaks in 2016, 2018, and 2020. Additionally, in 2018, the topotype VII, Lib-12 lineage of SAT2, was documented during outbreaks.[11]

Despite these incursions, serotype O has remained the most prevalent strain across Egypt, consistently causing outbreaks and posing ongoing challenges. Recently, a new lineage of serotype A, designated as FMDV-A-EgyAHRI-RL385-Ven-2022, emerged in Egypt, showing genetic similarities to strains circulating in Venezuela and Colombia. This novel strain presents a new threat to livestock health and national biosecurity, highlighting the evolving nature of FMDV in the country.[12]

Vaccination remains a cornerstone for controlling and preventing FMD, requiring frequent updates in vaccination strategies to address emerging strains. Studies have shown that vaccination reduces the incidence of FMD symptoms by approximately 70 % compared to unvaccinated controls, while booster vaccination further enhances protection, leading to a 90 % reduction in clinical cases. However, several environmental factors, such as temperature during vaccine storage and administration, significantly impact vaccine potency and efficacy.[13]

Monitoring post-vaccination serology is a crucial component of evaluating FMD vaccination programs. However, differences between the antigens used in diagnostic tests, vaccines, and circulating field viruses can influence the correlation between antibody titers and actual protection levels.[14]

This study aims to comprehensively assess FMD vaccine efficacy through both *in vitro* and *in vivo* studies. The research focuses on evaluating vaccine purity, antigenic matching, and immune response correlation to ensure effective protection. By comparing experimental and field data, the study seeks to provide a scientific basis for optimizing vaccination strategies, improving vaccine formulations, and enhancing long-term immunity in livestock.

Material and Methods

Evaluation of inactivated FMD vaccine in experimental animals

Virus and cells

FMDV A/Africa/G-IV, FMDV-A-Egy-AHRI-RL385-Ven-2022, O/EGY-4-2012, A/EGY/1/2012, SAT2/EGY-2012, and SAT2VII, Lib-12 were provided by the Strain Bank Department at Central Laboratory for Evaluation of Veterinary Biologics (CLEVB) for the assessment of existing inactivated FMDV vaccines. These strains were available in two forms: tissue culture -adapted virus and virulent virus, intended for use in the virus neutralization test (VNT) [9,12,15] and challenge tests involving experimentally vaccinated calves.

Baby hamster kidney cells (BHK-21), continuous cell line widely used for FMDV propagation, were specifically utilized in the VNT.[9,12,15] These cells were provided by the FMD vaccine production Department at the Veterinary Serum and Vaccine Research Institute (Abbassia, Cairo, Egypt).

Vaccine

The CLEVB meticulously selected a single batch (n=1) of an FMD vaccine based on its outstanding potency results. This batch was deliberately chosen to provide a comprehensive representation of all prevalent FMDV serotypes circulating in Egypt. The selection process aimed to ensure a robust and representative evaluation of vaccine efficacy against the diverse FMDV strains present in the region.

Selected batch (1): a locally produced commercial polyvalent oil-inactivated FMDV vaccine, formulated in Egypt using local isolate serotypes: A/Africa/G-IV, FMDV-A-Egy-AHRI-RL385-Ven-2022, O/EGY-4-2012, A/EGY/1/2012, SAT2/EGY-2012, and SAT2VII, Lib-12.

Experimental design

Animals were vaccinated according to the manufacturer's recommendations of the vaccine.

A. Calves

A total of 42 local-breed calves, aged 6 to 8 months, were used in the study. These calves were screened for the presence of specific antibodies against FMDV serotypes O/EGY-4-2012(O PanAsia), A/Africa/G-IV (A Africa), FMDV-A-Egy-AHRI-RL385-Ven-2022(A Venezuela), A/EGY/1/2012 (A Iran), SAT2/EGY-2012 (SAT2 GH), and SAT2VII, Lib-12 (SAT2 Libya) using the VNT[9,12,15] and confirmed seronegative.

Out of the 42 calves:

30 calves were vaccinated subcutaneously (S/C) with one field dose of the previously evaluated local commercial polyvalent oil-inactivated FMDV vaccine. These were divided into six challenge groups, each containing five calves.

12 calves served as a positive control group, with two calves assigned for each challenge strain.

B. Experimental groups

Group 1: five vaccinated calves were challenged with FMDV serotype O/EGY-4-2012.

Group 2: five vaccinated calves were challenged with FMDV serotype A/Africa/G-IV.

Group 3: five vaccinated calves were challenged with FMDV serotype FMDV-A-Egy-AHRI-RL385-Ven-2022.

Group 4: five vaccinated calves were challenged with FMDV serotype A/EGY/1/2012.

Group 5: five vaccinated calves were challenged with FMDV serotype SAT2/EGY-2012.

Group 6: five vaccinated calves were challenged with FMDV serotype SAT2VII, Lib-12.

Group 7 (positive control): twelve unvaccinated calves (two per strain) were challenged with the respective FMDV strains.

The design of the animal experiments in this study adhered to the guidelines outlined in the studies by Abousenna MS, et al.[9] and Nermeen GS, et al.[16]

Vaccine safety

All vaccinated animals were kept under clinical observation for any abnormalities. Injection sites were carefully examined for adverse effects, and in cases where visible or palpable reactions occurred, a detailed

description was recorded, as the study groups were not blinded.

Body temperature was monitored daily for 14 days post -vaccination and additionally 4 days prior to vaccination, continuing throughout the vaccine-efficacy study.

Sampling

Blood samples were collected before vaccination to confirm that all experimental calves were free from FMDV antibodies. Any calves testing positive for FMDV antibodies were excluded from the study.

Following vaccination, blood samples were collected weekly until 28 days post-vaccination and tested using the VNT[9,12,15] and the AsurDx FMD Multispecies Antibodies ELISA test kit to assess the immune response.

Virus neutralization test

The VNT[9,12] was performed using the microtiter neutralization technique on BHK-21 cells, following the method described by World Organization for Animal Health (WOAH).[15] The VNT antibody titer was considered protective when it reached ≥ 1.65 $\log_{10}$ Tissue Culture Infective $Dose_{50}$ ($TCID_{50}$), serving as a benchmark for vaccine efficacy.

AsurDx FMD Multispecies Antibodies ELISA Test Kit

The AsurDx FMD Multispecies Antibodies ELISA test kit is used to detect antibodies against non-structural proteins (NSP) in bovine serum samples. This test enables the differentiation between infected and vaccinated animals and also assesses the vaccine purity by detecting the presence of residual NSPs. The kit was obtained from BIOSTONE Animal Health, USA (Cat# 10040-05B).

Challenge test

On the 28th day post-vaccination, both vaccinated and control groups were relocated to the challenge room within the animal facility. Each vaccinated group (Groups 1–6) and their respective positive control animals (Group 7) were challenged with their corresponding FMDV strains, including O/EGY-4-2012, A/Africa/G-IV, FMDV-A-Egy-AHRI-RL385-Ven-2022, A/EGY/1/2012, SAT2/EGY-2012, and SAT2VII, Lib-12.

The challenge viruses were adjusted to a titer of 10^4 Bovine Infective $Dose_{50}$ (BID_{50})/0.2 mL and inoculated intradermolingually at two sites per animal. All calves, including vaccinated and control groups, were observed daily for 7 days for notable clinical signs, particularly ulcers on the tongue and feet, indicative of FMDV infection.

For the challenge test to be considered valid:

Positive control animals had to exhibit at least three feet ulcers.

Protection level was defined as ≥ 75 % (at least 3.75 out of five vaccinated animals protected from generalized foot infection).

The mean expectancy of protection (EPP) value of 75 % was used as an indicator of vaccine efficacy in protecting against the field strain, following the standards set by Nermeen GS, et al.[16] and WOAH.[15]

Animal care and welfare

All experimental animals underwent sedation before the challenge and during examinations. Infected animals received comprehensive veterinary care and medical treatment until full recovery, after which they were transferred to a designated post-experimental housing area. Animal handling and management followed the procedures outlined by Abousenna MS.[6]

Evaluation of inactivated FMD vaccine in field animals

Pre-vaccination sample collection

To evaluate the efficacy and purity of the inactivated FMD vaccine in field conditions, blood samples (n=600) were collected before vaccination from 30 animals per governorate. The targeted governorates (n=20) included: Gharbia, Menoufia, Port Said, Beheira, Sharqia, Suez, Fayoum, Beni Suef, New Valley, Dakahlia, Qalyubia, Ismailia, Damietta, Red Sea, Aswan, Assiut, Matrouh, Qena, Minya, and North Sinai.

The collected blood samples were analyzed using the AsurDx FMD Multispecies Antibodies ELISA test kit to differentiate between infected and vaccinated animals. Only seronegative animals (those without pre-existing antibodies against FMDV) were selected for vaccination to ensure an accurate evaluation of the vaccine's immunogenicity.

Animal vaccination

In each governorate, all seronegative animals (n=variable per governorate based on ELISA results) were selected for vaccination. These animals were administered a single field dose of the previously evaluated inactivated FMD vaccine, following the manufacturer's instructions. Each governorate received the same vaccine batch to maintain consistency in evaluation.

Post-vaccination blood sample collection

To assess the immune response and vaccine efficacy, blood samples were collected from vaccinated animals at:

One month post-vaccination

Two months post-vaccination

Four months post-vaccination

These samples were tested using the VNT[9,12,15] to measure and compare the neutralizing antibody titers over time, ensuring that the vaccine induced a protective immune response.

Vaccine purity assessment

At one month post-vaccination, an additional set of sera samples was collected from vaccinated animals in each governorate (n=20). These samples were tested using the AsurDx FMD Multispecies Antibodies ELISA test kit to detect antibodies against FMDV non-structural proteins (NSPs). This test was performed to confirm the absence of NSPs, ensuring that the vaccine was free from viral replication remnants and met the required purity standards.

Statistical analysis

A comparative evaluation was conducted to assess the relationship between experimental vaccine-induced VNT titers and field-measured VNT titers across 20 Egyptian governorates. Data from controlled experimental trials and longitudinal field surveillance were integrated for this analysis. Six different FMDV strains were included: O PanAsia, A Africa, A Iran, A Venezuela, SAT2 Libya, and SAT2 GH.

To account for variability in VNT titers measurements due to regional differences, a mixed-effects linear regression model was employed. The dependent variable was the mean field VNT titer, while the main fixed effect predictor was the experimental VNT titer at matching time points (1, 2, and 4 months post-vaccination). A random intercept was included for each governorate to account for between-region variability.

All statistical analyses were conducted using R version 4.3.1. The lme4 package was used for mixed-effects modeling (lmer function), and lmerTest was applied to compute p-values. Data processing and visualization were performed using the tidyverse suite.

Ethical approval for the animal experiments

The current study followed the Animal Research: Reporting of In-Vivo Experiments (ARRIVE) guidelines. All procedures involving animal use strictly adhered to the guidelines established by the Institutional Animal Care and Use Committee at the Agricultural Research Center (ARC-IACUC). Ethical approval for this study was obtained from the Committee (ARC-IACUC) approval No (ARC-CLEVB-56-24). The manuscript is considered compliant with bioethical standards in good faith.

No anesthesia or euthanasia protocols were employed for the animals involved in this study, as all animal-dependent methodological procedures were categorized as either no or low-pain procedures that can be ethically performed on a conscious and living animal.

Results

Evaluation of inactivated FMD vaccine in experimental animals

The inactivated polyvalent FMD vaccine induced strong humoral responses in experimental animals, as demonstrated by VNT titers and protection outcomes

(Table 1). At 28 days post-vaccination, all vaccine strains achieved VNT titers exceeding the protective threshold (1.65 log_{10} $TCID_{50}$), ranging from 1.98 for SAT2 GH to 2.40 for O PanAsia and A Venezuela. Titers continued to rise by 2 months post-vaccination, reaching peak levels for all strains, with the highest observed for A Venezuela (3.27) and O PanAsia (3.15). A gradual decline was noted by 4 months; however, titers for all strains remained above the protective threshold except for SAT2 GH, which decreased to 1.59.

Post-challenge protection correlated with VNT responses, showing 100 % protection for O PanAsia, A Africa, A Venezuela, and SAT2 Libya, while A Iran and SAT2 GH provided 80 % protection.

Evaluation of inactivated FMD vaccine in field animals

Differentiation between vaccinated and infected animals

Analysis of 600 field serum samples collected from 20 governorates using the AsurDx FMD Multispecies Antibodies ELISA test kit revealed variable proportions of negative and positive results indicative of vaccinated/ FMD-free and infected animals, respectively (Table 2). Negative results dominated across all governorates, ranging from 20 (66.7%) in Menoufia and Damietta to 26 (86.7%) in Ismailia and Assiut. Conversely, positive samples indicating natural infection were most frequent in Menoufia and Damietta (10 animals each, 33.3%) and least frequent in Ismailia and Assiut (4 animals each, 13.3%).

Intermediate levels of positive detection (5-9 animals, 16.7 - 30 %) were observed in most governorates, including Fayoum, Beni Suef, New Valley, and Red Sea. These findings highlight regional differences in natural exposure, likely influenced by variations in vaccination coverage, animal movement, and biosecurity practices.

Post-vaccination neutralizing antibody responses across Governorates

Pre-vaccination titers were uniformly below the protective threshold (≥ 1.65 log_{10} $TCID_{50}$), ranging from 0.00 to 0.30 log_{10} $TCID_{50}$ across all governorates (Table 3A). This indicates an absence of pre-existing immunity, with only sporadic low titers detected against O PanAsia and A Iran (0.30 log_{10} $TCID_{50}$).

Post-vaccination titers increased markedly (Table 3B). One month post-vaccination, mean titers increased markedly, reaching protective levels in most governorates for all strains, with the highest responses recorded against O PanAsia, A Africa, and SAT2 Libya. At 2 months, neutralizing titers peaked across all locations, averaging 2.69 - 2.97 log_{10} $TCID_{50}$, including strong responses against the antigenically diverse SAT2 GH strain.

Table 1. Evaluation of neutralizing antibody titers and protection levels in experimental animals following FMD vaccination.

FMD strains	***VNT			Protection level (%)
	28 day	2 months	4 months	
O PanAsia	*2.4	3.15	1.86	**100
A Africa	2.1	3	1.65	100
A Iran	2.1	2.88	1.8	80
A Venezuela	2.4	3.27	1.82	100
SAT2 libya	2.16	2.88	1.76	100
SAT2 GH	1.98	2.76	1.59	80

VNT: virus neutralization test.
*Cut-off titre for evaluating immunological protection afforded by vaccination ≥ 1.65 log_{10} $TCID_{50}$.
**Protection level (%) of challenge test ≥ 75%
***Neutralizing antibody titres using VNT.

Table 2. Field-based differentiation of FMDV infection and vaccination using AsurDx FMD Multispecies Antibodies ELISA test kit.

Governorate	No of samples	*Negative (vaccinated or free)	Positive (infected)
Gharbia	30	22 (73.3 %)	8 (26.7 %)
Menoufia	30	20 (66.7 %)	10 (33.3 %)
Port Said	30	21 (70.0 %)	9 (30.0 %)
Beheira	30	21 (70.0 %)	9 (30.0 %)
Sharqia	30	22 (73.3 %)	8 (26.7 %)
Suez	30	23 (76.7 %)	7 (23.3 %)
Fayoum	30	25 (83.3 %)	5 (16.7 %)
Beni Suef	30	25 (83.3 %)	5 (16.7 %)
New Valley	30	24 (80.0 %)	6 (20.0 %)
Dakahlia	30	22 (73.3 %)	8 (26.7 %)
Qalyubia	30	22 (73.3 %)	8 (26.7 %)
Ismailia	30	26 (86.7 %)	4 (13.3 %)
Damietta	30	20 (66.7 %)	10 (33.3 %)
Red Sea	30	21 (70.0 %)	9 (30.0 %)
Aswan	30	24 (80.0 %)	6 (20.0 %)
Assiut	30	26 (86.7 %)	4 (13.3 %)
Matrouh	30	25 (83.3 %)	5 (16.7 %)
Qena	30	25 (83.3 %)	5 (16.7 %)
Minya	30	23 (76.7 %)	7 (23.3 %)
North Sinai	30	23 (76.7 %)	7 (23.3 %)

*Results are expressed as the number and percentage of negative (vaccinated or FMD-free) and positive (infected) animals per governorate. Positive: inhibition percentage > 50%; Negative: inhibition percentage < 50 %.

Table 3A. Mean neutralizing antibody titers (log_{10} $TCID_{50}$) using virus neutralization test against FMDV strains pre-vaccination across different governorates.

Govs	No of samples	*Mean antibody titer using VNT					
		Pre-vaccination					
		O PanAsia	A Iran	A Africa	A Venezuela	SAT2 Libya	SAT2 GH
Gharbia	22	0	0.3	0.15	0.3	0.15	0
Menoufia	20	0.3	0.3	0	0.3	0	0.3
Port Said	21	0.14	0.15	0.3	0.13	0.3	0.16
Beheira	21	0	0.16	0	0.075	0.14	0.13
Sharqia	22	0.21	0	0	0.3	0.14	0.16
Seuz	23	0	0	0.21	0.17	0.13	0.15
Fayoum	25	0	0.07	0	0.3	0.17	0.13
BeniSuef	25	0.3	0.18	0.13	0.18	0.14	0
NewVally	24	0.14	0.16	0	0.3	0.12	0.16
Dakahlia	22	0.15	0.08	0	0.15	0.18	0
Qalyubia	22	0.15	0.19	0.3	0.3	0.16	0.17
Ismailia	26	0	0.3	0.3	0	0.12	0.15
Damietta	20	0.3	0	0.15	0.3	0.15	0.16
Red Sea	21	0	0.23	0.075	0.07	0	0.3
Aswan	24	0.12	0.21	0.082	0.14	0.3	0
Assiut	26	0.3	0.15	0	0.17	0.13	0.14
Matrouh	25	0	0.13	0.3	0.14	0	0.3
Qena	25	0.14	0.3	0.17	0.14	0.3	0.15
Minya	23	0.13	0.13	0	0.17	0.14	0
North Sinia	23	0	0.24	0.073	0.21	0.3	0.16

VNT: virus neutralization test.
*Cut-off titre for evaluating immunological protection afforded by vaccination using VNT ≥ 1.65 log_{10} $TCID_{50}$.
Govs: governorates.

Table 3B. Mean neutralizing antibody titers (log_{10} $TCID_{50}$) using virus neutralization test against FMDV strains post-vaccination across different governorates.

Govs	No of samples	Mean antibody titer using VNT																	
		Months post-vaccination																	
		1						2						4					
		O PanAsia	A Iran	A Africa	A Ven	SAT2 Libya	SAT2 GH	O PanAsia	A Iran	A Africa	A Ven	SAT2 Libya	SAT2 GH	O PanAsia	A Iran	A Africa	A Ven	SAT2 Libya	SAT2 GH
Gharbia	22	1.77	2	1.83	1.85	1.85	1.72	2.73	2.77	2.74	2.69	2.54	2.45	1.54	1.56	1.51	1.53	1.53	1.47
Menoufia	20	1.95	2.05	1.85	1.96	1.78	1.75	2.79	2.75	2.78	2.75	2.53	2.48	1.59	1.55	1.52	1.57	1.55	1.49
Port Said	21	2	2	1.83	2.12	1.94	1.81	2.82	2.8	2.79	2.76	2.52	2.5	1.61	1.6	1.49	1.59	1.53	1.5
Beheira	21	1.86	2.03	1.81	1.85	1.82	1.79	2.76	2.78	2.75	2.72	2.55	2.49	1.56	1.59	1.49	1.53	1.52	1.46
Sharqia	22	2.3	2.03	1.88	2.15	1.96	1.94	2.97	2.82	2.79	2.78	2.59	2.53	1.64	1.6	1.51	1.61	1.54	1.47
Seuz	23	2.06	2.01	1.82	2.03	1.95	1.85	2.95	2.83	2.81	2.76	2.55	2.51	1.52	1.61	1.48	1.49	1.52	1.46
Fayoum	25	2.04	1.89	1.83	2.05	1.92	1.88	2.91	2.79	2.79	2.73	2.51	2.48	1.59	1.59	1.49	1.56	1.51	1.45
BeniSuef	25	2.03	1.78	1.85	2.06	1.89	1.83	2.89	2.74	2.77	2.71	2.5	2.46	1.58	1.57	1.51	1.54	1.54	1.49
NewVally	24	2.05	2.01	1.85	2.04	1.92	1.84	2.91	2.87	2.81	2.76	2.52	2.51	1.58	1.56	1.52	1.55	1.54	1.5
Dakahlia	22	2.07	2.08	1.83	2.09	1.95	1.85	2.92	2.86	2.85	2.75	2.51	2.5	1.57	1.59	1.51	1.54	1.53	1.48
Qalyubia	22	1.85	1.98	1.79	1.81	1.78	1.77	2.86	2.84	2.73	2.69	2.48	2.44	1.53	1.59	1.52	1.51	1.56	1.51
Ismailia	26	2.03	1.89	1.83	1.95	1.92	1.83	2.72	2.8	2.84	2.75	2.54	2.51	1.57	1.6	1.5	1.54	1.56	1.52
Damietta	20	2.1	2	1.85	2.15	1.96	1.91	2.92	2.83	2.89	2.78	2.57	2.53	1.61	1.6	1.51	1.58	1.53	1.48
Red Sea	21	2.11	2.09	1.84	2.09	1.93	1.91	2.89	2.86	2.86	2.74	2.54	2.51	1.61	1.63	1.51	1.59	1.51	1.45
Aswan	24	2.05	2.01	1.81	2.03	1.86	1.86	2.86	2.81	2.83	2.72	2.53	2.48	1.59	1.6	1.48	1.55	1.52	1.47
Assiut	26	2	2.01	1.81	1.95	1.82	1.85	2.85	2.8	2.85	2.71	2.49	2.47	1.58	1.61	1.49	1.55	1.51	1.46
Matrouh	25	2.03	2.05	1.83	2.03	1.91	1.82	2.86	2.81	2.83	2.73	2.51	2.48	1.58	1.61	1.51	1.54	1.51	1.45
Qena	25	2.04	2.01	1.83	2.01	1.95	1.82	2.84	2.81	2.83	2.72	2.52	2.45	1.59	1.6	1.51	1.56	1.53	1.47
Minya	23	2.08	2.02	1.84	2.03	1.96	1.84	2.86	2.82	2.84	2.73	2.52	2.45	1.58	1.61	1.51	1.55	1.54	1.47
North Sinia	23	2.07	2	1.82	2.07	1.92	1.84	2.88	2.81	2.88	2.72	2.5	2.46	1.57	1.61	1.49	1.53	1.52	1.45

VNT: virus neutralization test.
*Cut-off titre for evaluating immunological protection afforded by vaccination using VNT ≥ 1.65 log_{10} $TCID_{50}$
Govs: governorates.
A Ven: A Venezuela.

By 4 months, titers declined slightly but generally remained above the protective threshold, indicating sustained immunity. Protection was particularly robust and persistent for O PanAsia, A Africa, and SAT2 Libya strains.

The evaluated polyvalent inactivated FMD vaccine elicited strong and durable neutralizing antibody responses, maintaining protective levels for at least 4 months under field conditions.

Assessment of vaccine purity based on AsurDx FMD Multispecies Antibodies ELISA test kit for NSPs antibodies one month after vaccination

One month following administration of an inactivated FMD vaccine, cattle across 20 Egyptian governorates were evaluated for the presence of antibodies against NSPs using the AsurDx FMD Multispecies Antibodies ELISA test kit. As presented in Table 4, the percentage of NSP- animals ranged from 80.0 % to 90.9 %, with the highest observed in Qalyubia (90.9 %) and Menoufia (90%).

Statistical analysis

The mixed-effects regression model revealed a significant positive association between experimental and field virus-neutralizing antibody titers across all strains and governorates ($p < 0.001$). The fixed-effect slope (β) was 0.71.

Strain-specific analysis demonstrated variation in predictive strength. The strongest correlation was observed for O PanAsia ($\beta = 0.76$, $p < 0.001$), whereas SAT2 GH showed the weakest, though still significant, correlation ($\beta = 0.65$, $p = 0.003$).

Table 4. Evaluation of FMD vaccine purity one month post vaccination using the AsurDx FMD Multispecies Antibodies ELISA test kit to detect NSP antibodies in cattle across Egyptian Governorates.

Govs	No of samples	AsurDx FMD Multispecies Antibodies ELISA test kit		**Purity (%)
		*No of NSP- (pure)	No of NSP+ (not pure)	
Gharbia	22	18	4	81.8
Menoufia	20	18	2	90
Port Said	21	17	4	80.1
Beheira	21	18	3	85.7
Sharqia	22	19	3	86.3
Seuz	23	20	3	86.9
Fayoum	25	20	5	80
BeniSuef	25	21	4	84
NewVally	24	21	3	87.5
Dakahlia	22	19	3	86.4
Qalyubia	22	20	2	90.9
Ismailia	26	21	5	80.7
Damietta	20	16	4	80
Red Sea	21	18	3	85.7
Aswan	24	21	3	87.5
Assiut	26	22	4	84.6
Matrouh	25	21	4	84
Qena	25	20	5	80
Minya	23	20	3	86.9
North Sinia	23	20	3	86.9

*NSP- (pure): animals testing negative for NSP antibodies (inhibition percentage < 50 %).
NSP+ (not pure): animals testing positive for NSP antibodies (inhibition percentage > 50 %).
**Purity (%) = (NSP- animals/Total tested animals) × 100.

Discussion

This study systematically evaluated the immunogenicity, protective efficacy, and purity of a locally produced polyvalent inactivated FMD vaccine, formulated to target six major circulating FMDV strains in Egypt, including newly emergent variants such as A/ Africa/G-IV and A Venezuela. By conducting both controlled experimental and extensive field evaluations, a comprehensive assessment of vaccine performance was achieved under varied conditions, enhancing the understanding of its potential in national FMD control efforts.

In the controlled experimental phase, the vaccine demonstrated strong immunogenicity across all tested strains. By 28 days post-vaccination, VNT titers surpassed the critical protective threshold of 1.65 $\log_{10}$ $TCID_{50}$, aligning with WOAH standards.[15] Peak titers were observed at 2 months post-vaccination, followed by a gradual decline at 4 months. This immune response pattern is consistent with prior experimental vaccine evaluations conducted in Egypt, such other studies,[12,16] affirming the vaccine's ability to induce a strong and durable antibody response against multiple FMDV serotypes.

Challenge studies provided further insight into strain-specific protective outcomes. Complete protection (100 %) was observed against O PanAsia, A Africa, A Venezuela, and SAT2 Libya strains. In contrast, partial protection (80 %) was recorded against A Iran and SAT2 GH strains, despite achieving initial serological thresholds. Notably, SAT2 GH-specific neutralizing antibody titers fell below the protective limit by the 4 month, a finding that resonates with previous reports of antigenic divergence in SAT2 field isolates.[6] This divergence likely impacts vaccine-induced protection, emphasizing the importance of continuous monitoring for antigenic variation in circulating strains.

The partial protection against A Iran, despite protective titers, suggests that neutralization assays, while predictive, do not always guarantee clinical protection, particularly for genetically or antigenically evolving strains.[9] These findings highlight the importance of complementing serological evaluation with *in vivo* challenge studies to capture the full spectrum of vaccine -induced protection.

Several observations emerged from the experimental data. All vaccinated calves exhibited strong anamnestic responses following challenge, demonstrating effective immunological priming regardless of minor titer fluctuations. While most vaccine components maintained protective titers up to 4 months, the reduced immunity against SAT2 GH indicates that booster vaccination strategies may be necessary to sustain field-level protection against this strain. Furthermore, the variability in protection between strains underscores the need for regular vaccine updates based on antigenic surveillance and the inclusion of emerging variants in vaccine formulations.

Transitioning from controlled trials to field conditions, the vaccine's performance was evaluated across 20 Egyptian governorates involving 600 cattle. Pre-vaccination screening using the AsurDx FMD Multispecies Antibodies ELISA test kit established a rigorously seronegative study cohort, ensuring that subsequent immune responses could be attributed solely to vaccination rather than natural exposure. The observed seronegative rates, ranging from 66.7 % to 86.7 %, revealed regional differences in prior FMDV exposure, reflecting the complex epidemiological landscape in Egypt. This rigorous approach aligns with WOAH guidelines for field vaccine trials[15] and builds upon established methodologies,[17] while specifically addressing Egypt's diverse epidemiological landscape. The pre-trial screening proved particularly valuable when interpreting post-vaccination results, as the confirmed seronegative status at enrollment allowed clear differentiation between vaccine-induced immunity and natural infections occurring during the study period.

Post-vaccination assessments confirmed the vaccine's capacity to induce protective neutralizing antibody responses across all governorates. By one month post-vaccination, mean VNT titers for all six strains had reached or exceeded protective levels. Peak antibody responses were documented at 2 months (2.69–2.97 $\log_{10}$ $TCID_{50}$), consistent with the immunogenic profiles observed in the experimental arm. Over time, a gradual decline in titers was observed, particularly notable

against SAT2 GH by 4 months post-vaccination. Nevertheless, the durability of protection against the majority of strains supports the vaccine's utility in maintaining herd immunity under field conditions.

These field observations align closely with reports from other FMD vaccine trials,[18] which indicated similar antibody kinetics for a heptavalent oil-adjuvanted vaccine, noting peak titers around 60 days post-vaccination with sustained protection thereafter. Likewise, the experimental data from Pirbright Institute's VNT analysis of Aphthovac-4 vaccinated calves showed similar patterns - high titers against most test strains (2.6-3.15 log10 for serotypes A and O), though certain lineages (A/Irn/25/18, O/Cathay) exhibited reduced neutralization, mirroring our field observations of differential protection across strains and governorates.[19]

Vaccine purity assessments provided additional confirmation of vaccine quality. NSP-specific antibody detection through AsurDx FMD Multispecies Antibodies ELISA test kit revealed that over 80 % of vaccinated animals remained NSP-negative one month post-vaccination across all governorates which met the minimum acceptable threshold for purity, as per the standards outlined by WOAH,[15] which states that vaccines should not elicit NSP antibody responses in the absence of field virus exposure. As NSPs are associated with active viral replication, their presence in vaccinated animals would indicate either contamination with live virus or incomplete removal of NSPs during vaccine production. However, NSPs are typically eliminated during the ultrafiltration stage of inactivated vaccine manufacture, and animals vaccinated with high-purity vaccines should not mount an NSP-specific antibody response. Thus, the detection of NSP-negative (NSP−) status in the majority of animals serves as a proxy for the vaccine's purity and proper inactivation. This result is consistent with the WOAH Differentiating Infected from Vaccinated Animals (DIVA) standard,[15] where high vaccine purity minimized the risk of confounding diagnostic outcomes. Such vaccine purity is essential for effective disease surveillance and the implementation of DIVA strategies, allowing clear differentiation between infected and vaccinated animals.

The relationship between laboratory-based experimental outcomes and real-world field performance was evaluated using mixed-effects linear regression modeling. A strong positive correlation ($\beta = 0.71$, $p < 0.001$) was identified between experimental VNT titers and field VNT titers across all strains, indicating that experimental data can serve as a reliable predictor of field immunogenicity. However, notable regional variability was observed (governorate-level variance = 0.06), suggesting that local factors such as herd management practices, vaccination handling, environmental stressors, and possible silent viral circulation influence immune responses. This finding underscores the need for regionally tailored immunosurveillance systems and field evaluations to ensure optimal vaccine performance under diverse epidemiological conditions. This approach aligns with best practices for biological inference in complex epidemiological systems, as recommended.[20]

The variability observed in field responses highlights the challenges of FMD control in endemic regions, where viral evolution, biosecurity differences, and animal management practices contribute to heterogeneity in vaccine performance. Strain-specific differences, particularly the reduced persistence of immunity against SAT2 GH, indicate that booster vaccinations may be necessary in high-risk areas. Future improvements should focus on incorporating newly circulating strains, optimizing adjuvants, and exploring novel vaccine platforms to enhance long-term immunity. These findings emphasize the importance of continuous vaccine evaluation, proactive strain matching, and integrated surveillance to support effective FMD control strategies.

Conclusions

This study demonstrated that the locally produced polyvalent inactivated FMD vaccine was safe, immunogenic, and effective under experimental and field conditions across 20 Egyptian governorates. It achieved complete protection against O PanAsia, A Africa, A Venezuela, and SAT2 Libya strains, and partial protection against A Iran and SAT2 GH strains. Although antibody titers declined against SAT2 GH by 4 months, the vaccine maintained overall protective levels and met international purity standards. A strong

correlation between experimental and field immunogenicity supports its reliability. These findings highlight the importance of updated polyvalent vaccines and tailored surveillance in controlling FMD in endemic regions like Egypt.

Conflict of interest

The authors declare that there is no conflict of interest.

Author's contributions

Nermeen Gouda-Shafik: conceptualization, validation, and investigation.

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Amal Abd El-Moneim-Mohamed: methodology, validation, and formal analysis.

Sara El Sawy-Ahmed: methodology, validation, and investigation.

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Fady Abd El-Mohsen Shasha: methodology, formal analysis, investigation.

Samir A. Nassif: investigation.

All authors have read and agreed to the published version of the manuscript.

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Seguimiento de la eficacia de la vacunación contra la fiebre aftosa basado en comparaciones experimentales y de campo: de la evaluación a la protección

Resumen

La fiebre aftosa sigue siendo endémica en Egipto debido a la coexistencia de múltiples serotipos. La vacunación es la piedra angular de los esfuerzos de control; sin embargo, la aparición de nuevas cepas requiere una evaluación periódica de la eficacia de la vacuna. En este estudio se evaluó una vacuna polivalente inactivada contra la fiebre aftosa, producida localmente, dirigida a seis serotipos prevalentes, tanto en condiciones experimentales como de campo. Se vacunó experimentalmente a 42 terneros seronegativos y posteriormente se les expuso a cepas homólogas para evaluar la protección. Simultáneamente, se llevó a cabo una evaluación de campo en 600 bovinos distribuidos en 20 gobernaciones egipcias. Las respuestas inmunitarias se midieron mediante pruebas de neutralización del virus y el estuche ELISA AsurDx FMD Multispecies Antibodies, mientras que la pureza de la vacuna se confirmó mediante la detección de anticuerpos contra proteínas no estructurales. Todos los animales vacunados experimentalmente desarrollaron títulos de anticuerpos neutralizantes por encima del umbral de protección ($\geq$1,65 $\log_{10}$ $TCID_{50}$), logrando una protección del 100 % contra cuatro cepas. En el campo, el ganado vacunado mostró títulos protectores sostenidos hasta 4 meses después de la vacunación, aunque se observó una disminución de los títulos contra la cepa SAT2 GH. Las pruebas de proteínas no estructurales confirmaron la pureza de la vacuna, con más del 80 % de los animales dando negativo en todas las provincias estudiadas. El análisis de regresión de efectos mixtos mostró una fuerte correlación positiva entre los títulos de las pruebas de neutralización del virus experimentales y de campo (β = 0,71, $p < 0,001$), lo que respalda la extrapolación de los resultados de laboratorio a las condiciones de campo. En general, se demostró que la vacuna era segura, inmunogénica y ampliamente eficaz en diversas condiciones. El seguimiento continuo de las cepas circulantes y las actualizaciones oportunas de la vacuna son esenciales para mantener un control eficaz de la fiebre aftosa en regiones endémicas como Egipto.

Palabras clave: fiebre aftosa; potencia de la vacuna; cobertura de vacunación; pruebas serológicas; estudio comparativo.

Received: July 28, 2025 *Accepted: October 31, 2025*