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Potency of a commercial inactivated trivalent foot-and-mouth disease vaccine in local goats of the Diyala Governorate, Iraq

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Foot-and-Mouth Disease remains one of the most economically devastating infections affecting livestock producers globally. This study evaluated the immunogenicity and field efficacy of a commercial inactivated trivalent vaccine containing serotypes O, A, and Asia-1 in the local goat population of the Diyala Governorate, Iraq, focusing on assessing how host sex and pregnancy status modulate the resulting humoral immune response. A total of 178 clinically healthy local breed goats, comprising 100 females and 78 males aged between 7 and 36 months, were immunized with a single 1 mL dose administered subcutaneously. Paired blood samples were collected immediately before vaccination (Day 0) and at Day 28 post-vaccination to capture peak antibody synthesis. Serological analysis was performed using a commercial solid-phase competitive enzyme-linked immunosorbent assay kit, with antibody levels expressed as percentage inhibition, where a threshold of greater than or equal to 50 % was defined as protective immunity. Evaluation of baseline samples revealed a pre-vaccination seropositivity rate of 15.2 % (27/178), confirming that the virus is endemic to the region. Following immunization, the overall protective seropositivity rate increased significantly to 89.3 % (159/178) by Day 28 ($p<0.001$), and the mean percentage inhibition values more than doubled across all three viral strains. Host sex exerted no statistically significant influence on vaccine responsiveness ($p=0.541$), yielding comparable seropositivity rates of 87.2 % for male and 90.8 % for female cohorts. Conversely, pregnancy status imposed a substantial physiological limitation on vaccine-induced immunogenicity. Pregnant goats exhibited a significantly lower protective seropositivity rate of 82.1 % compared to 97.3 % in non-pregnant animals ($p=0.026$). Furthermore, gestation significantly suppressed antibody synthesis levels for serotypes O ($p=0.001$) and Asia-1 ($p=0.002$). In conclusion, while the commercial trivalent vaccine is safe and highly potent, pregnancy-induced immunosuppression significantly reduces its protective efficacy. These findings suggest that small ruminants must be integrated into national control programs, and vaccination campaigns should be strategically timed during non-pregnant phases, such as the early dry period or prior to the breeding season, to optimize herd immunity.

Keywords: vaccine potency, goats, enzyme-linked immunosorbent assay, pregnancy, immunity, Foot-and-Mouth Disease.

Ефективність комерційної інактивованої тривалентної вакцини проти ящуру у місцевих кіз провінції Діяла, Ірак

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Ящур залишається однією з найбільш економічно збиткових інфекцій, що завдає збитків виробникам тваринницької продукції в усьому світі. У цьому дослідженні оцінено імуногенність та ефективність комерційної інактивованої тривалентної вакцини, що містить серотипи O, A та Asia-1, на місцевій популяції кіз у провінції Діяла, Ірак. Основну увагу приділено аналізу того, як стать і стан вагітності тварин модулюють поствакцинальну імунну відповідь. Загалом 178 клінічно здорових кіз місцевої породи (100 самок і 78 самців віком від 7 до 36 місяців) імунізували одноразовою дозою об'ємом 1 мл підшкірно. Парні зразки крові відбирали безпосередньо перед вакцинацією (день 0) та на 28-й день після неї для фіксації піку синтезу антитіл. Серологічний аналіз проводили з використанням комерційного набору для твердофазного імуноферментного аналізу, а рівень антитіл виражали у відсотках інгібіції, де захисним порогом вважали показник вище або рівний 50 %. Оцінка вихідних зразків виявила початковий рівень серопозитивності 15,2 % (27/178), що підтверджує ендемічність вірусу в регіоні. Після імунізації загальний показник захисної серопозитивності суттєво зріс до 89,3 % (159/178) на 28-й день ($p<0,001$), а середні значення відсотка інгібіції зросли більш ніж удвічі для всіх трьох вірусних штамів. Стать тварин не чинила статистично значущого впливу на реактивність вакцини ($p=0,541$), демонструючи зіставні рівні серопозитивності: 87,2 % у самців та 90,8 % у самок. Навпаки, стан вагітності накладав суттєві фізіологічні обмеження на вакциноіндуковану імуногенність. Кітні кози мали значно нижчий рівень захисної серопозитивності – 82,1 % порівняно з 97,3 % у кітних тварин ($p=0,026$). Крім того, кітність суттєво пригнічувала рівень синтезу антитіл до серотипів O ($p=0,001$) та Asia-1 ($p=0,002$). Хоча комерційна тривалентна вакцина є безпечною та висококонкурентною, викликана кітністю кіз імуносупресія суттєво знижує її захисну ефективність. Отримані результати свідчать про необхідність інтеграції дрібних жуйних тварин до національних програм контролю, а кампанії з вакцинації слід стратегічно планувати на невагітні фази репродуктивного циклу, такі як ранній сухостійний період або період безпосередньо перед початком сезону парування, для оптимізації популяційного імунітету.

Ключові слова: ефективність вакцини, кози, імуноферментний аналіз, кітність, імунітет, ящур.

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Introduction

Among various livestock diseases, Foot-and-Mouth Disease (FMD) remains one of the most economically devastating for livestock producers. FMD is caused by the FMD virus (FMDV), a member of the *Picornaviridae* family, which targets cloven-hoofed animals [1]. FMDV comprises seven immunologically distinct serotypes that are highly mutable, which significantly aggravates disease control [2]. Because immune responses induced by vaccination are not cross-protective between different strains, vaccine formulations used in FMD vaccination campaigns are based on BEI-inactivated viral particles and usually contain more than one virus strain [3].

In Iraq, serotype A was officially recorded for the first time in 1952, followed by Asia-1 and serotype O in later years [4]. Disease control measures currently applicable in Iraq focus on mass vaccination using inactivated FMDV serotypes O, A, and Asia-1 [5]. Evaluating immunological control and the field response to these inactivated FMD vaccines is vital. The World Organisation for Animal Health sets the benchmark for control measures based on the PD_{50} (50 % Protective Dose) test [6]. Generally, the Solid-Phase Competitive ELISA (SPCE) serological method and a >50 % inhibition concentration (%IH) value [7] for neutralizing antibodies are used to determine the correlation and efficacy of protective vaccines [8, 9]. The standard control threshold is set at a 50 % SPCE inhibition level.

Most FMD control programs view cattle as the primary targets. However, small ruminants, such as sheep and goats, also play a critical role in FMD epidemiology. These animals can act as maintenance hosts, showing mild or subclinical signs while efficiently spreading the virus [10]. Despite their importance, literature reporting the outcomes of vaccination in goats, especially under unique physiological conditions, remains limited.

Immunological factors, such as sex and pregnancy, are important in modulating the immune response but are often overlooked during field vaccination campaigns [11]. During pregnancy, immunological tolerance is enhanced to allow gestation to progress without the maternal immune system rejecting the fetus. This physiological shift can potentially impede the host's response to vaccination [12].

Iraq imports vaccines from various global sources. While these imported formulations consistently pass standard quality control benchmarks, their real-world field performance in the local small ruminant population requires continuous evaluation.

The aim of the study

The aim of this study was to evaluate the immune responsiveness of goats in the Diyala Governorate to a commercial trivalent FMD vaccine and to assess the impact of sex and pregnancy status on the resulting immune response.

Materials and methods

Study Area, Animals, and Design

The research was conducted in the Diyala Governorate, an agricultural region in eastern Iraq. For this cross-sectional study, a geographical spread was achieved by randomly selecting 178 local breed goats from six smallholder farms in Al-Muqdadiya. The sample included 100 females and 78 males aged between 7 and 36 months. All animals were clinically healthy and had not been vaccinated against FMD for at least six months prior to the study. The experimental design involved mass vaccination followed by paired serological sampling.

Vaccine and Vaccination Protocol

A commercially available, inactivated, oil-adjuvanted, trivalent FMD vaccine containing serotypes O, A, and Asia-1 was used. The vaccine was stored appropriately at 4 °C. Each goat received a single 1 mL dose administered subcutaneously in the neck region.

Sample Collection

Blood samples (5 mL) were collected from the jugular vein into plain vacutainer tubes immediately before vaccination (baseline sample, Day 0) and at Day 28 post-vaccination (to capture the peak antibody response). Serum separation was performed by centrifuging the samples for 15 minutes at 3,000 × g. The collected sera were then aliquoted and stored frozen at -20 °C until analysis.

FMD Serology

Serological analysis was performed using a commercial ELISA kit (PrioCHECK™ FMDV Type O/A/Asia-1 antibody ELISA, Thermo Fisher Scientific) in strict accordance with the manufacturer's instructions. This assay is a prescribed test for FMD seromonitoring according to the World Organisation for Animal Health (WOAH) guidelines. The test operates on the principle of competitive binding to an FMDV antigen-coated plate. The results were calculated using the percentage inhibition (%IH) formula:

$$\%IH = \left[1 - \left(\frac{OD_{\text{sample}}}{OD_{\text{negative control}}}\right)\right] \times 100$$

Any sample recording a %IH≥ 50 % was considered positive for protective antibodies.

Statistical Analysis

Data analysis was conducted using GraphPad Prism software (version 9.5.1). The Shapiro-Wilk test was applied to assess data normality. Chi-square tests (χ^2) were used to determine the statistical significance of categorical data (seropositivity rates). For continuous data (mean %IH) from two related groups (Day 0 vs. Day 28), a paired t-test was utilized. Unpaired t-tests were used to compare two independent groups (male vs. female, pregnant vs. non-pregnant). A p-value of <0.05 was considered statistically significant.

Results and discussion

Clinical observations and vaccine safety

The observation period lasted for 72 hours post-vaccination for all animals. Only mild, transient swelling was noted at the injection site in some goats; this local reaction completely resolved within 48 hours without any medical treatment. These observations indicate a favorable safety profile of the commercial vaccine under field conditions.

Overall seropositivity and antibody response

Evaluation of baseline immunity showed that prior to vaccination, 15.2 % (27/178) of the goats were seropositive, indicating previous FMDV exposure. Following vaccination, the overall seropositivity rate increased significantly to 89.3 % (159/178), demonstrating a robust immune response (χ^2=125.6, p<0.001).

The detailed dynamics of seroconversion and antibody levels for each specific serotype are summarized in ***Table 1***.

Table1

Overall seroconversion (count, %) and mean antibody response (mean %IH ± SD) in goats (n=178)

Parameter	Pre-vaccination (Day 0)	Post-vaccination (Day 28)	*p*-value
Seropositivity rate	15.2 % (27/178)	89.3 % (159/178)	< 0.001 (χ^2 test)
Serotype O	32.1±11.5	78.4±9.8	< 0.001 (Paired *t*-test)
Serotype A	28.5±13.2	72.1±11.3	< 0.001 (Paired *t*-test)
Serotype Asia-1	30.8±10.9	75.9±10.5	< 0.001 (Paired *t*-test)

As shown in ***Table 1***, the mean percentage inhibition values for all three evaluated serotypes showed a highly significant increase from baseline to post-vaccination levels. Following immunization, a substantial growth in antibody levels was observed across all strains. The highest relative increase in antibody response was recorded for serotype A, which demonstrated a 2.53-fold growth compared to its baseline level (p<0.001). A similar pronounced expansion was also captured for serotypes Asia-1 (p<0.001) and O (p<0.001), which increased by 2.46-fold and 2.44-fold, respectively. These findings demonstrate that the commercial trivalent vaccine induces a robust and balanced humoral immune response, actively stimulating antibody production against all three viral serotypes within the local goat population.

Influence of sex on immune response

To optimize research resources and diagnostic kit utilization, a representative sub-sampling approach was employed, selecting a cohort of 120 goats (55 males and 65 females) for serological evaluation. Analysis of the post-vaccination parameters demonstrated that both subgroups achieved high levels of protective antibodies by Day 28. The comparative breakdown of seropositivity rates and mean antibody levels between the sexes is presented in ***Table 2***.

Table 2

Post-vaccination immune response (mean %IH±SD) in male and female goats (total n = 120, selected from a herd of 178 animals)

Parameter	Male goats (n = 55)	Female goats (n = 65)	*p*-value
Seropositivity rate	87.2 % (48/55)	90.8 % (59/65)	0.541 (χ^2 test)
Serotype O	77.6±10.2	79.1±9.5	0.403 (Unpaired *t*-test)
Serotype A	71.3±11.8	72.8±10.9	0.471 (Unpaired *t*-test)
Serotype Asia-1	75.2±11.0	76.5±10.1	0.503 (Unpaired *t*-test)

As shown in ***Table 2***, statistical analysis confirmed that host sex did not exert a significant influence on vaccine-induced humoral protection. The minor variations observed in seropositivity rates between the male and female cohorts lacked statistical significance (p=0.541). Similarly, the mean percentage inhibition values across all three evaluated viral strains remained highly comparable between the groups, with no statistically verifiable deviations detected for serotypes O (p = 0.403), A (p = 0.471), or Asia-1 (p = 0.503). These results indicate that the commercial trivalent vaccine provides uniform immunological protection in goats regardless of sex.

Influence of pregnancy on immune response

To further investigate internal physiological modulations, the female cohort (n = 65) was stratified by gestation status into pregnant (n = 28) and non-pregnant (n = 37) subgroups. The comparative data regarding the impact of pregnancy on the vaccine's immunogenicity are summarized in ***Table 3***.

Table 3

Post-vaccination immune response (mean %IH ± SD) in female goats by pregnancy status (total n = 65)

Parameter	Pregnant goats (n = 28)	Non-pregnant goats (n = 37)	*p*-value
Seropositivity rate	82.1 % (23/28)	97.3 % (36/37)	0.026 (χ^2 test)
Serotype O	74.8±10.5	82.3±7.1	0.001 (Unpaired *t*-test)
Serotype A	68.9±11.8	75.6±9.2	0.051 (Unpaired *t*-test)
Serotype Asia-1	72.1±10.8	79.7±8.3	0.002 (Unpaired *t*-test)

As shown in ***Table 3***, physiological status significantly affected the host's immune responsiveness. Non-pregnant animals exhibited a highly pronounced seropositivity rate compared to the pregnant group (p=0.026). Furthermore, pregnancy status significantly suppressed antibody production levels. For serotypes O and Asia-1, non-pregnant goats achieved a highly significant expansion in antibody levels (p=0.001 and p=0.002, respectively) compared to their pregnant counterparts. A similar downward trend was observed for serotype A, though it marginally missed the traditional statistical significance threshold (p=0.051).

This study emphasizes the significance of the physiological status of animals, contributes to the ongoing research on Foot-and-Mouth Disease (FMD) vaccination, and documents its field application in goats in Iraq. The overall seroconversion rate of 89.3 % and the substantial increases in the percentage inhibition (%IH) values during the post-vaccination period confirm the high immunogenicity of the vaccine, as highlighted in the World Organisation for Animal Health (WOAH) reference documents and by other authors [9]. The baseline figures pertaining to FMDV diagnostics, which revealed that 15.2 % of the animals were seropositive pre-vaccination, indicate that FMDV remains endemic to the Diyala region. These data point to the critical importance of vaccinating small ruminants, which frequently serve as viral reservoirs [4, 11].

As pointed out in the FAO-UN monthly report on FMD status in 2016, Iraq is included in the Middle East and West Eurasia epidemiological zone according to its geographical location. This region shares environmental similarities with ecosystems characterized by high virus circulation, where FMDV serotypes O, A, and Asia-1 traditionally dominate [13, 14]. Consequently, there must be continuous tracking of circulating FMD virus serotypes to systematically evaluate and select the most suitable vaccine formulations, rather than relying strictly on historical data or previously dominant serotypes distributed in the area [15].

One of the more notable findings of this research was the lack of a significant difference between the sexes regarding their immune response to the vaccine. This is evidenced by the highly comparable post-vaccination seropositivity rates of 87.2 % in males and 90.8 % in females ($p=0.541$). This demonstrates that both the vaccine antigen and the adjuvant system are robust enough to effectively activate the immune systems of both male and female goats, eliciting uniform mean percentage inhibition values across all tested strains ($p>0.05$). This outcome is highly valuable for field applications, as it streamlines the mass vaccination process and removes the practical need for any sex-based vaccination protocols.

The most important finding from this study remains the diminished antibody response observed in pregnant goats. This phenomenon is attributed to the immunosuppressive tendencies associated with pregnancy, which are well-documented in other species and closely tied to high levels of progesterone and cortisol that promote a T-helper 2 (Th2) immune response. This physiological shift centers around fostering maternal immune tolerance [12, 15] in order to preserve the pregnancy. However, in doing so, it can significantly limit the mother's response to novel antigens, such as those present in a vaccine. This mechanism explains the lower post-vaccination seropositivity rate (82.1 % vs. 97.3 %, $p=0.026$) and suppressed mean %IH values for serotypes O ($p=0.001$) and Asia-1 ($p=0.002$) found in our pregnant cohort.

Our findings are in line with recent research in livestock indicating reduced immunogenicity of several other pregnancy-associated vaccines [11, 16]. This factor is critical in terms of disease control. Kids born to vaccinated mothers depend entirely on transferred colostral antibodies for immunological protection during their early days of life. If the maternal response to vaccination is insufficient, these kids will ingest inadequate passive immunity through the colostrum. Thus, while field observations confirm it is safe to vaccinate goats during pregnancy, the procedure is unlikely to provide the desired epidemiological benefits for the offspring.

A limitation of this study is the measurement of humoral immunity at a single peak timepoint (Day 28). Future longitudinal studies tracking antibody persistence in pregnant versus non-pregnant animals are highly required. Additionally, the parallel assessment of cell-mediated immunity would help in obtaining a more comprehensive picture of the complex immune modulation that occurs during pregnancy [17].

The inactivated trivalent FMD vaccine is highly effective in goats, with both sexes responding equally. However, pregnancy significantly limits antibody production and reduces protective immunity rates. Therefore, animal physiological status must be considered to optimize vaccination timing.

Based on these findings, the following control measures are recommended:

Inclusion of Small Ruminants: formally integrate sheep and goats into national FMD programs to build herd immunity and break the transmission cycle.

Strategic Timing: schedule immunization during non-pregnant phases, preferably during the early dry period or just before breeding [18–21], to maximize host immune response [16].

Booster Evaluation: assess whether pregnancy booster doses can elevate maternal antibody titers and enhance passive colostral immunity for newborns.

Active Surveillance: maintain continuous virological monitoring to ensure precise antigenic matching between imported vaccine strains and circulating field variants.

Conclusions

The inactivated trivalent FMD vaccine demonstrated high immunogenicity in the local goat population, driving a significant increase in the overall seropositivity rate from a baseline of 15.2 % (27/178) to 89.3 % (159/178) by Day 28 post-vaccination ($p<0.001$). Following immunization, mean %IH values more than doubled across all strains, showing a highly significant 2.53-fold growth for serotype A, 2.46-fold for serotype Asia-1, and 2.44-fold for serotype O ($p<0.001$). Host sex does not exert a statistically significant influence on vaccine efficacy ($p=0.541$), yielding highly comparable protective antibody levels and final seropositivity rates of 87.2 % (48/55) for male and 90.8 % (59/65) for female goats. Conversely, pregnancy imposes a substantial physiological limitation on vaccine responsiveness, resulting in a significantly lower protective seropositivity rate of 82.1 % (23/28) in pregnant goats compared to 97.3 % (36/37) in non-pregnant counterparts ($p=0.026$). Gestation significantly suppresses antibody levels for serotypes O ($p=0.001$) and Asia-1 ($p=0.002$), with a similar downward trend captured for serotype A ($p=0.051$), confirming that animals should ideally be vaccinated during non-pregnant phases.

DECLARATIONS

Ethical Statement

The authors declare that the conducted studies fully comply with generally accepted norms of humane treatment of animals and the principles of bioethics. Ethical approval was officially granted by the Bioethics Committee of the Department of Internal and Preventive Medicine at the College of Veterinary Medicine, University of Diyala, Iraq (Protocol No. 22 dated 03.09.2025). To minimize animal stress and discomfort, all handling, restriction, and blood sampling procedures were performed in strict accordance with standard veterinary guidelines and current international principles of animal welfare.

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Conflict of Interest

The author states that there is no conflict of interest.

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Declaration of AI and AI-assisted technologies

The author declares that no generative AI or AI-assisted technologies were used during the preparation, writing, translation, or language polishing of this manuscript. The final content was fully authored and reviewed by the researcher, who takes sole responsibility for the work.

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