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# The efficacy of FMD vaccine prepared from recently locally isolated FMD virus using Ictyolane 18 oil adjuvant.

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**ABSTRACT**

FMD is an important viral disease that affects animals with cloven hooves. Inactivated oil adjuvant FMD vaccines are widely used in prophylactic vaccination programs for protection of animals and for safe and effective immunization. In this study, we prepared a bivalent inactivated vaccine form recently isolated FMDV strains A/Africa G4/2022 and A/ Venezuela/2022 serotypes using different ratio of Ictyolane 18 oil adjuvant and evaluate quality and immunogenicity safety of vaccine. Laboratory evaluation of the vaccine using calves that vaccinated intramuscularly (I/M) with the prepared bivalent FMD vaccines (group 1 using the vaccine emulsion 50 % Ictyolane 18 oil with 50% antigen and group 2 using the vaccine emulsion 60 % Ictyolane 18 with 40% antigen). Serum samples collected from animals that had received vaccination were nine months. Serum Neutralization Test (SNT) and the ELISA method were used to monitor the humeral immune responses. The findings showed that the protective level was reached by vaccinated calves at the second to third week following vaccination (WPV) and persisted up to nine months following vaccination. The findings demonstrate that the inclusion of the two ratio of Ictyolane 18 in Cattle receive an early and prolonged high-specific protective immune response from the FMDV vaccine. Finally, we recommended to use Ictyolane 18 as a potential safe and very cost-effective adjuvant with an equal ratio of FMD antigen for the cattle FMD vaccination.

## 1. INTRODUCTION

Foot and mouth disease (FMD) is a contagious viral illness that affects animals with cloven feet animals. (Orsel et al., 2007). A single-stranded positive-sense RNA virus belonging to the genus *Aphthovirus* and the family *Picornaviridae* is the cause. The FMDV has seven immunologically distinct serotypes, which are as follows: Asia1, O, A, C, SAT1, SAT2, and SAT3 (OIE, 2021). One FMDV serotype infection or vaccination does not result in protection against other FMDV serotypes and may not fully protect against various subtypes within the same serotype.(Paton et al., 2005). In addition to contaminated feed, polluted vehicles, and diseased persons, it can spread dramatically through the air. Thus, once an outbreak starts, the economic impact is severe. Egypt has had many outbreaks of the FMDV since 1950, making it one of the endemic African nations (Mousa et al., 1974).

FMDV strain serotype A/Africa/G-VII.Ken-05 was introduced to Egypt through live animals importation (Abdel-Rahman et al., 2006; Knowles et al., 2007; WRLFMD, 2013). The other strain related to serotype A (A/Asia/Iran-05BAR-08) was introduced in 2010 (WRLFMD, 2013). Serotype A topotype Africa Genotype IV was isolated in Egypt in 2012-2020 (Soltan et al., 2017; Hassan et al., 2022). The significance of the need for border controls and quarantines have been reinforced by the current FMDV epidemiology and the spread of serotype O, A, and SAT-2 in Egypt. (EL-Bagoury et al., 2022). FMDV serotype A-African-Genotype IV in circulation in Egypt during 2022 (Shahein et al., 2022).

One of the most practical and efficient ways to put an end to FMD epidemics is through FMDV vaccines (Paton et al., 2009). The effective use of high-quality vaccinations has been a key element in the control and/or elimination of FMD (Allende et al., 2003). Many types of adjuvants are employed in veterinary vaccines, mineral oil-based adjuvants used for inactivated FMD vaccines (Park et al., 2016). FMD vaccine formulated with the oil adjuvant, showed potential in the FMD enzootic region (Khorasaniet al., 2016). The choice of a suitable adjuvant is the most crucial element in determining the effectiveness of these vaccinations. To make sure FMD epidemics receive a fast and suitable response.

The objective of this study was to evaluate the efficacy of the newly developed bivalent inactivated FMD vaccine using Ictyolane 18 oil adjuvant that was prepared from recently isolated serotype A FMDV strains in 2022.

## 2. MATERIAL AND METHODS

*Ethics Declarations*

The authors of this study followed the guidelines established by the animal welfare committee and the protocols used in

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the research were approved by the Research Ethics Committee at the Faculty of Veterinary Medicine, Benha University. Additionally, the study was registered at the national level (BUFVTM 17-01-23).

*2.1. FMD Virus strains (A/Africa G4/2022 and A/Venezuela 2022):*
Recently isolated FMD virus serotype A strains (A/Africa G4/2022 and A/Venezuela 2022) of cattle origin were obtained from FMD Department at Veterinary Serum and Vaccine Research Institute, (VSVRI) Abasia, Egypt. It was typed at FMD Department in VSVRI, Abasia, Egypt. These viruses were adapted on BHK cell line and were stored at -70 °C till being used in vaccine preparation and also as reference viruses for SNT and ELISA.

*2.2. Virus propagation and titration:*
BHK cell line was sub-cultured using growth medium utilizing the approach outlined by Macpherson and Stocher (1962). It was supplied from Iezlar Institute for Animal Health, Italy. The FMDV vaccine seed strains were propagated in confluent monolayer sheet of BHK-21 cells. The harvested viruses were collected within 17-24 hrs. when the cytopathic effect (CPE) appeared.
BHK-21 was used for virus propagation and titration (OIE Manual, 2012). The virus titer was expressed as $\log_{10}TCID_{50}$/ ml as described by Reed and Muench (1938). The virus titer was not less than 7 $\log_{10}$ $TCID_{50}$ /ml (OIE, 2017).

*2.3. Ictyolane 18 oil adjuvant:*
This is a mineral oil-based adjuvant form a water-in oil-in water emulsion without the need for surfactant. It was used for the preparation of FMD oil-based vaccine to be administrated subcutaneously or intra-muscular. It was obtained from ICTYO DEV, Bethany, France.

*2.4. Preparation of the inactivated bivalent FMD vaccine:*
*2.4.1. Inactivation of FMDV*
The clarified FMD virus serotype A strains (A/Africa G4/2022 and A/Venezuela 2022) were inactivated using binary ethylene-imine as 1% of the 0.1 M stock solution (BEI) in the 0.2 N Na OH (8gm/ 1L DDW) prepared solution (Bahnemann, 1975). Then 2% (40gm $Na_2$ $S_2$ $O_3.5H_2O$ was dissolved in 100mL DDW. It was added at 2% to the inactivated virus to neutralize the action of BEI.

*2.4.2. Formulation of inactivated bivalent FMD vaccines with Ictyolane 18 oil adjuvant:*
It was carried out using cell culture adapted inactivated FMDV serotype A strains (A/Africa G4/2022 and A/Venezuela 2022) at a titer of 7 $\log_{10}$ $TCID_{50}$/ml and Ictyolane 18 oil adjuvant according to manufacturing company, the vaccine was formulated by adding slowly the aqueous phase containing equal volumes of the seed virus strains to the adjuvant at the required percent (50% antigens and 50 % Ictyolane 18 oil for group 1 vaccine and 40% antigen and 60 % Ictyolane 18 with for group 2 vaccine) with stirring at 350 rpm for 5 minutes at 30ºC then mix thoroughly and followed by brief mixing cycle for 24 hrs. at 4 °C.

*2.5. Quality control of the prepared vaccines:*
*2.5.1. Sterility test:*
It was used to ensure that there were no bacterial or fungal contaminations in the vaccine. The tested vaccine was cultured on nutrient agar, thioglycolate broth, and Sabouraud's dextrose agar to ensure its sterility (CFR, USA, 1986; OIE 2000).

*2.6.2. Safety test for the formulated FMD vaccine:*
*2.6.2.1. Safety test for inactivated viruses on BHK cell culture:*
A volume of 0.1 ml of each inactivated virus strain before addition of adjuvant was inoculated on BHK21 clone 13 monolayer cell line (25 cm/2 prescription tissue culture flask). The inoculated cells were incubated for 72 hrs at 37 °C. Positive and negative control cells were conducted. The culture was examined daily for CPE (rounding of cells, granulation of cytoplasm, detachment from the tissue culture flask surface).Completion of virus inactivation is ensured by absence of CPE (CFR, USA, 1986; OIE 2000).

*2.6.2.2. Safety test for inactivated viruses on baby mice:*
Before addition of adjuvant, a volume of 0.1 ml of each inactivated virus strain was inoculated by intraperitoneal route in baby mice (three to five days old). The inoculated mice were recognized for signs of disease or death for over one week. Positive and negative control mice (7 animals/ group) were conducted. Completion of virus inactivation is ensured by absence of signs of disease or death (CFR, USA, 1986; OIE 2000).

*2.6.2.3. Safety of prepared vaccine in susceptible calves:*
The safety test of the prepared vaccines was done by intra-dermo-lingual inoculation of 1 ml of the vaccine in 10 sites of the tongue of susceptible calves. The vaccines considered safe when no local or general lesions appeared and rise of temperature was recorded over one week (Henderson, 1970; CFR, USA, 1986; OIE 2000).

*2.7 Experimental design:*
A total number of 16 naive calves (clinically sound and devoid of antibodies to FMD viral strains, as shown by SNT) were used for evaluation of the humoral immune response to the prepared inactivated bivalent FMD vaccines with Ictyolane 18 oil adjuvant as follow:
Group 1: 5 calves vaccinated with FMD vaccine 50 %Ictyolane 18.
Group 2: 5 calves vaccinated with FMD vaccine 60 %Ictyolane 18.
Group 3: 3 calves were used as negative control (non-vaccinated)
Group 4: 3 calves were used for safety test.
Vaccines were inoculated in animals in 2ml/dose by intramuscular route.

*2.8. Evaluation of the immune response:*
Blood samples were collected from calves at days zero, 7th, 14th, 21st, 28th, then every month till the end of experiment. The serum samples were rendered inactive by 30 minutes of heating at 56°C. The immune response was evaluated through SNT described by Ferreira (1976) and ELISA according to OIE (2012) using FMDV vaccine strains (A/Africa G4/2022 and A/Venezuela 2022).

## 3. RESULTS

3.1. Sterility of the prepared vaccines:
The prepared vaccines were sterile and free from any contaminating bacteria and fungi.

3.2. Safety of the prepared FMD vaccines:
The seed FMDV strains were completely inactivated and safe as indicated by absence of CPE on BHK cells and

absence of signs and deaths of mice following inoculation of the inactivated virus in them. The prepared FMD vaccines using both vaccine emulsion 50 % Ictyolane 18 oil with 50% antigen and 60 % Ictyolane 18 with 40% antigen were safe to use after being inoculated in calves with no local lesions or general symptoms.

3.3. Evaluation of the humoral immune response to the prepared FMD vaccines:

The mean serum antibody titer for the prepared bivalent inactivated FMD vaccine using 50 % Ictyolane 18 oil emulsion with 50% antigen started to increase from the first week post vaccination (WPV) and reached the protective titer from the second WPV using SNT and ELISA (~~Table 1 and~~ Figure 1).

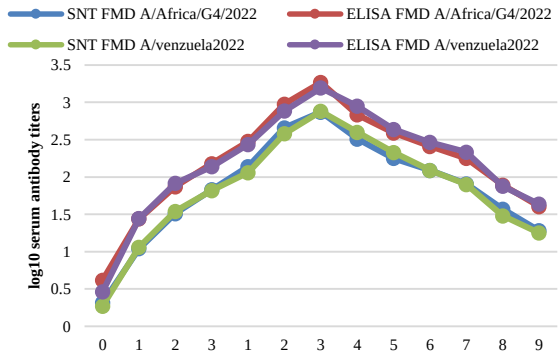


Figure 1 Tracking level and duration of serum antibody titers in calves vaccinated with bivalent FMD vaccines with 50% Ictyolane 18 oil adjuvant.

The mean serum antibody titer for the prepared bivalent inactivated FMD vaccine using 60 % Ictyolane 18 oil emulsion with 40% antigen started to increase from the first week post vaccination (WPV) and reached the protective titer from the third WPV using SNT and ELISA (Figure 2).

The mean serum antibody titer for both prepared FMD vaccines using 50 % Ictyolane 18 oil emulsion with 50% antigen and 60 % Ictyolane 18 oil emulsion with 40% antigen reached the highest titers at 3 months post vaccination (MPV) and continued in the protective level for 8 MPV using SNT and ELISA ( Figures 1 & 2).

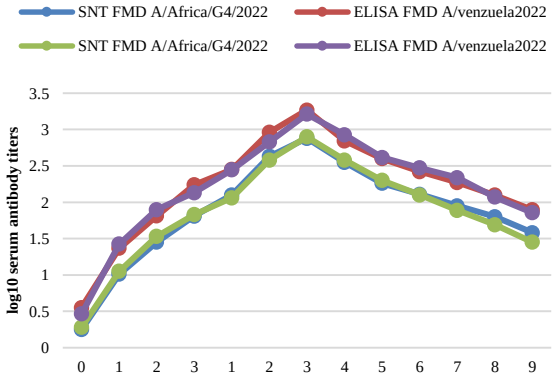


Figure 2 Tracking level and duration of serum antibody titers in calves vaccinated with bivalent FMD vaccines with 60% Ictyolane 18 oil adjuvant.

## 4. DISCCUSION

Foot and Mouth Disease (FMD) is a serious disease brought on by the FMDV that has a significant negative economic impact (Orsel et al., 2007).

In order to successfully restrict the illness in endemic regions and ensure that vaccination of animals limits the spread of FMD, it was thought that controlling FMD in animals was crucial.

The vaccinations tested negative for any pathogenic or non-pathogenic bacteria in the culturing sterility test. Also, safety of inactivated virus and prepared vaccines were in figures (1 & 2) indicated that no viable viral residues of all serotypes used in vaccine preparation, so the vaccines were safe to use. These findings were in line with the recommendation of OIE (2000) that the FMD vaccination be devoid of any live viruses.

In this research, we looked at how Ictyolane 18 affected the immune response that was protective and specific against FMD. The result summarized in figures (1 & 2) the results revealed that SNT and ELISA for FMD vaccine with Ictyloan18 oil adjuvant gave the protective serum antibody levels at 2$^{nd}$ - 3$^{rd}$ week post vaccination.

According to Wisniewski et al. (1972) and OIE (2009), who noted that the SNT measures those antibodies that neutralize the infectivity of FMD virion, SNT titers for FMD vaccinations go hand in hand with the findings obtained.

The mean antibodies titers of FMD vaccines continues with protective level till 8th MPV (examined with SNT and ELISA test). These ELISA obtained results were in parallel correlation with those obtained by SNT. A positive correlation between ELISA and virus neutralization titers for sera (Hamblin et al., 1986). The protective level was 1.5 $log_{10}$ by means of SNT which equivalent to by means of ELISA (OIE, 2009). Results supported also by Batista et al. (2010) and Fakhry and Assem (2014) who observed that adjuvant increased antibody synthesis and helped the vaccination function more efficiently. FMD vaccine adjuvanted with Ictyolane 18 showed satisfactory results in the manner of safety and potency test through their evaluation with A/Africa/G4/2022) and A/venzuela2022 strains. The bivalent FMD-Ictyolane vaccine induces higher level of antibodies and long period of immunity.

## 5. CONCLUSIONS

From the presented results we notice that the use of Ictyolane 18 adjuvant was safe and induced long-term higher immune response to FMD vaccine in cattle when used in different ratio first one 50% ictyolane18 : 50% antigen and second one 60% ictyolane18 : 40% antigen. Finally, we recommended the use of Ictyolane 18 as an ideal vaccination adjuvant that is extremely economical when used with equal ratio of antigen and oil specially FMDV.

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