



Research Article

Efficacy of a Commercial Local Trivalent Foot and Mouth Disease (FMD) Vaccine against Recently Isolated O-EA3

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ABSTRACT

Foot and Mouth Disease (FMD) is a highly infectious disease in cloven- hoofed animals, the vaccination is widely used to control. Recently the FMDV toptotype O-EA3 had been isolated that differs from the previous toptotype Middle East-South Africa (ME-SA) with lineage Panasia2 (O Panasia2) that was prevalent in Egypt from 2010 to 2012. This study was designed to detect and characterize the recently circulating field isolate strain of FMDV in Egypt antigenically, and furthermore detect the efficacy of the existing local commercial vaccine against the recently isolated strain of FMDV. Five calves were vaccinated by local commercial inactivated FMDV vaccine; sera were obtained weekly till 28th day post vaccination and tested by SNT. The virulent circulating virus O-EA3 was isolated and full identified by AHRI while the propagation and titration in cattle tongue were carried out in CLEVB to adjust the challenge dose, then the challenge had been carried out for the vaccinated calves by local commercial vaccine at 28th day post vaccination. It was found that the SNT showed protective neutralizing serum antibody titer (1.2 log₁₀) started from 3rd week post vaccination with 1,56 log₁₀ titer against FMDV type O in vaccinated calves, while the challenge test showed that the protection level was 100% against O-EA3 in vaccinated calves with local commercial vaccine, Therefore, it appeared that the antigenicity of the type O viruses circulating in the region has not changed greatly, and the vaccines of Middle East origin (PanAsia2) can also be used against O-EA3 to control the disease, and there is no need to vaccine update.

Key words: Foot and Mouth Disease (FMD), O-EA3

INTRODUCTION

Foot and mouth disease (FMD) is a highly infectious, economically devastating disease and remaining a globally important livestock animal disease affecting cloven-hoofed animals (Parida, 2009), which results in serious production losses and is considered a major constraint to international trade of livestock and their products from FMD endemic countries causes severe economic hardship to farmers.

Foot and mouth disease virus is the prototype member of the genus Aphthovirus of the family Picornaviridae and exists in seven immunologically and serologically distinct serotypes: (Euroasiatic serotypes A, O, C, and Asia1 and South African Territories [SAT] serotypes SAT1, SAT2 and SAT3) (Carrillo *et al.*, 2005).

Egypt suffered from many FMD outbreaks since 1950 and onwards. In 2012–2013, A serotype of Asian toptotype related to Iran genotype was reported (Sobhy *et al.*, 2014). O serotype has a long history of causing regular outbreaks in Egypt till now, although the regular obligatory vaccination in whole Egypt governorates (Mandour *et al.*, 2013; EL-Bayoumy *et al.*, 2014).

The attempts to control of FMDV outbreaks have been depended on routine vaccination of livestock (Bergmann *et al.*, 2005). Nevertheless, the vaccination may not provide the optimal protection against circulating field strains, so this study was designed to detect and characterize the recently circulating field strain of FMDV in Egypt antigenically with furthermore detection the efficacy of the existing local commercial vaccine against the recently isolated strain of FMDV.

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MATERIALS AND METHODS

Virus: FMD Serotype O topotype East-Africa 3 was isolated and identified in AHRI by RT-PCR (Naglaa *et al.*, 2017) and it was received by CLEVB to be used in current inactivated FMD vaccine evaluation. The supplied viruses were tissue culture adapted to be used for (SNT) and virulent for (challenge test).

Cell line: Baby Hamster Kidney (BHK-21) cell line was supplied by FMD Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. The cells were grown and maintained according to Macpherson and Stocker (1962). It was used for FMD virus adaptation, propagation, titration and serum neutralization test (SNT).

Vaccine: Local commercial trivalent oil inactivated FMD vaccine produced in VSVRI Egypt and prepared from local isolate serotypes O/EGY/4/2012, A/EGY/1/2012 and SAT2/EGY/2/2012 had been selected for this study. The existing vaccine was previously evaluated with satisfactory results by CLEVB.

Calves and experimental design: Nine calves (local breed) of six to eight months old of about 200 – 300 kg body weight were allotted into 3 groups and kept in separate breeding stables. The sera from these calves were previously screened by SNT for the presence of specific antibodies against FMD virus and did not reveal any specific antibodies (sero-negative). They were allotted into three groups as follow:

Group (1): two calves were used for challenge virus titration.

Group (2): five calves were vaccinated with previously evaluated local commercial FMD vaccine.

Group (3): two calves left as non-vaccinated group (control positive for challenge test).

Serum Neutralization Test (SNT): The bovine sera for groups (2) and (3) were used to measure the efficacy of FMD vaccination against FMD virus type O-EA3, the test was performed by using the microtechnique as described by Ferriera (1976).

Virus titration in calves tongue: Infectivity titration was carried out for FMD virus strain O-EA3, which was used in the challenge test. Serial tenfold dilution in Hank's balanced salt solution was prepared from FMD virus type O-EA3 to be titrated. The dilutions were inoculated in the tongue of calves (2 calves), dividing the tongue of calves by using Indian ink into rows, where each dilution was inoculated in a row of five sites intradermolingually using 0.1 ml for each site. The inoculated tongue sites examined carefully and records daily for three days post inoculation as vesicles formation were counted in each row, reported by (Cox *et al.*, 2007), to avoid rupture of these vesicles, a tranquilizer was injected to cattle before tongue examination. The virus titer was calculated and expressed as Log₁₀ (Bovine infective dose) BID₅₀/ml according to (Karber, 1931).

Challenge test: Both vaccinated and control calves were challenged after measuring of antibody titre at 28th day post vaccination with 0.3ml 10⁴ BID₅₀ of FMD virus strain O-EA3 via intradermolinguual inoculation then observed daily for symptoms of FMD for 7 days. The animals showing symptoms were subjected to virus re-isolation. The control positive animals must show at least 3 feet to consider the validity of the test (OIE, 2012).

Comparing results: This work was repeated Using different two batches of the local commercial inactivated FMDV vaccine in corresponding to the same condition of current work with comparing results.

RESULTS

Estimation of humoral immune response of vaccinated calves (Group 2) showed that protective neutralizing serum antibody titer (1.2 log₁₀) started from 3rd week post vaccination with 1.56 log₁₀ titer against O in vaccinated calves as shown in Table 1 and Figure 1. The titer of FMD virus serotype O-EA3 in calves tongue (Group 1) was found to be 10⁶ BID₅₀/ml, as shown in Table 2. Determination of protection level for O serotype of the vaccine in vaccinated calves (Group 2) with local commercial vaccine using challenge test against O-EA3 virus, showed that the protection level was 100%, while the control positive calves (Group 3) showed lesions in tongue and 4 feet, as shown in Table 3 and Figure 2.

Table 1: FMD type O serum neutralizing antibody titer in calves group 2 vaccinated T.

Weeks post Vaccinated animals	*Log ₁₀ /SNT titers weeks post vaccination				
	0 WPV	1 WPV	2 WPV	3 WPV	4 WPV
1	0.0	0.6	0.9	1.2	1.8
2	0.3	0.9	1.2	1.5	2.1
3	0.3	0.9	1.35	1.8	2.4
4	0.3	0.9	1.2	1.8	2.1
5	0.3	0.9	1.2	1.5	1.8
Mean	0.24	0.84	1.17	1.56	2.04
Control	0.3	0.3	0.3	0.3	0.3

*WPV= week post vaccination.

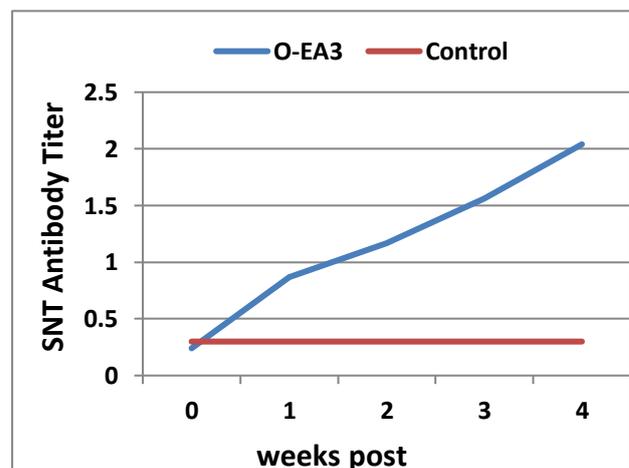


Fig. 1: FMD serum Neutralizing antibody titre vaccinated calves with local commercial inactivated FMD vaccine against FMDV O-EA3.



Fig. 2: Inspected clinical signs appeared on infected animals post challenge test.

Table 2: FMD virus type O-EA3 titer used in the challenge test in calves tongue.

FMDV Serotype	Infectivity titer
O-EA3	10 ⁶ BID ₅₀ /0.1 ml

*BID₅₀/ml: Titer of infective FMDV in calves tongue calculated using the formula Karber, (1931).

Table 3: Characteristic FMD lesions after challenge with O-EA3 in cattle (group2) Vaccinated with local commercial inactivated FMD vaccine.

Vaccinated animals	*Lesions of challenge test after 7 days				
	Tongue	Fore limbs		Hind limbs	
		Left	Right	Left	Right
1	+ve	- ve	- ve	- ve	- ve
2	- ve	- ve	- ve	- ve	- ve
3	- ve	- ve	- ve	- ve	- ve
4	- ve	- ve	- ve	- ve	- ve
5	- ve	- ve	- ve	- ve	- ve
Control 1	+ ve	+ve	+ ve	+ ve	+ ve
Control 2	+ve	+ ve	+ ve	+ ve	- ve
Protection %	100 %				

*Erosions in tongue and ulcers in inter-digital space.

DISCUSSION

FMD molecular epidemiological studies can determine the variation and genetic relationship among the circulating FMDV serotypes in Egypt. This genetic variation can partially explain persistent occurrence of outbreaks even with obligatory governmental vaccination (EL- Bayoumy *et al.*, 2014; Soltan, *et al.*, 2017). In 2014- 2016 FMD outbreaks, it was found that the FMDV isolates included toptotype East Africa-3 (EA-3) that differs from the previous toptotype Middle East-South Africa (ME-SA) with lineage Panasia2 (O Panasia2) that was prevalent in Egypt from 2010 to 2012 and also, different from the vaccinal strains that belongs to ME-SA toptotype (Rady *et al.*, 2014;Soltan, *et al.*, 2017).

The control of FMDV disease mainly depends on availability of matching vaccines that can be selected based on epidemiological information and serological cross-reactivity of bovine post- vaccinal serum (BVS) with circulating viruses. In addition, availability of sufficient doses of vaccines of good quality and potency is also equally important (Balinda *et al.*, 2010; Wekesa

et al., 2015). Trivalent vaccines are currently in use in Egypt for FMD control, but recently was recorded genetic variation between vacinal strains and circulating field isolates which impetus us to investigate the efficacy of the existing local commercial vaccine against the recently isolated strain FMDV O-EA3 using serum neutralization test (SNT) and challenge test.

The humoral immune response in vaccinated calves (group 2) with local commercial vaccine (O PanAsia 2) against FMDV type O-EA3 using SNT showed that protective neutralizing serum antibody titer (1.2 log₁₀) started from 3rd week post vaccination with 1,56 log₁₀ titer against O in vaccinated calves (OIE, 2012), all results of SNT presented in Table (1) and Figure (1) agree with (Katie *et al.*, 2017) who recorded that O/PanAsia-2 and O/Manisa vaccines revealed border protection against East African serotype O viruses, even though they genetically belong to the ME-SA toptotype.

The protection level for O serotype (PanAsia 2) of the vaccine in vaccinated calves (Group 2) with local commercial vaccine using challenge test against O-EA3 virus after screening of antibody titre at the 28th day post vaccination, showed that, all the result above of challenge test presented in Table (3) confirmed the serological cross-reactivity of bovine post-vaccinal serum (BVS) with circulating virus O-EA3. The relevant results had been compared to different two vaccine batches where they had been evaluated by challenge test using O-EA3 and the protective level showed 100% and 80% protection which confirmed the relevant results, From the discussed results we can conclude that the local commercial inactivated FMDV vaccine (O- PanAsia2) is potent and effective against circulating FMDV O-EA3, Therefore, it appeared that the antigenicity of the type O viruses circulating in the region has not changed greatly, and the vaccines of Middle East origin (PanAsia2) can also be used against O-EA3 to control the disease, and there is no need to vaccine update.

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