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RESEARCH ARTICLE

Evaluation of the efficacy of an inactivated FMD vaccine and its role in prevention of persistence and transmission of FMDV by Sheep.

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Abstract

A prepared inactivated bivalent FMD vaccine induced protective serum neutralizing antibody titers against both types of FMDV (A and O) at 14th day and up to either 21 days or 7 months post vaccination in sheep. It gave a protection with challenge either after 21 days or 7 months post vaccination with no rise in body temperature and no detection of FMDV (using ELISA) in oro-pharyngeal fluid samples collected from sheep for one month post challenge. Non-vaccinated sheep showed elevated body temperature and positive detection of FMDV in their oro-pharyngeal fluid samples for up to 5–8 months post challenge using ELISA and elevated serum neutralizing antibody titers that persisted for longer duration for both types A and O FMDV as detected by SNT. Non-vaccinated sheep could transmit infection to calves in contact and FMDV was detected using ELISA in calves tissue samples. Our results reflected the importance of highly potent FMD vaccine to inhibit local virus replication and prevent spread of FMDV by sheep.

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Introduction

Foot and Mouth disease (FMD) is one of the most wide spread diseases affecting cloven footed animals especially cattle, pigs and sheep with detrimental effects on meat and milk production, rather than convalescent animals became carriers and source of infection (Ibrahim, 2011). The disease is caused by a virus member of family Picornaviridae, genus Aphthovirus including seven main serotypes (O, A, C, SAT1, SAT2, SAT3, and Asia), as well as numerous intratypic strains (Meyer et al., 1991). In Egypt, where the disease is highly endemic, prophylactic vaccination against FMDV is the only mean of control (Moussa et al., 1984, Abed El- Rahman et al., 2006, Faraget et al., 2006, 2008 and 2009 and Ibrahim, 2011). Sheep are domestic animals that live in almost all cattle farms and play an important role in maintaining and transmitting FMDV, being a potential source of new outbreaks (Salt, 1998 and Laila et al., 2004). So, this experiment was designed to evaluate efficacy of a potent inactivated bivalent FMD vaccine and study its role in prevention of local replication, persistence and transmission of FMDV by sheep.

2. MATERIAL AND METHODS

2.1. Virus strains:

Locally isolated FMDV strains type A/Egypt/2006 and O1/3/93, confirmed by FMD world reference laboratory (WRL), Pirbright, UK, were kindly obtained from FMD vaccine research department, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo. Both virus strains were adapted for 8 passages on BHK₂₁ cell line and titrated according to Reed and Muench (1938).

2.2. Cell Culture:

Baby Hamster Kidney cells (BHK₂₁) were kindly obtained from FMD vaccine research department, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo. It were grown and maintained according to Macpherson and Stocker (1962), and used for virus propagation, titration, serum neutralization test and vaccine production.

2.3. Experimental Animals:

2.3.1. *Sheep:*

A total of 33 local breed sheep, 50-70 kg body weight and 1 – 2 years old were used to test safety and to evaluate efficacy of the vaccine.

2.3.2. *Calves:*

Twenty three, local breed calves of 6 – 8 months old and 200 – 300 kg body weight calves were used in testing safety of the prepared vaccine, titration of FMDV types (A and O), challenge of vaccinated and control non vaccinated sheep by contact with calves infected with 10^4 Bovine Tongue Infective Dose 50% (BTID₅₀) of virulent homologous virus strain, type A FMD virus and type O FMD virus, respectively and finally for studying the role of non-vaccinated sheep in transmission of FMDV to the in contact free non-vaccinated calves.

2.3.3. *Guinea Pigs:*

Fifteen healthy adult albino male guinea pigs of approximately 400-500 grams body weight were used for adaptation of FMD virus types A/Egypt/2006 and O1/3/93 in guinea pigs then testing potency of the prepared FMD vaccine by calculation of Protective Dose 50% (PD₅₀).

2.4. *Samples:*

2.4.1. *Serum samples:*

Sera were collected from vaccinated groups of sheep weekly then every two weeks till challenge time, and from unvaccinated groups of sheep after challenge time for one year. It stored at -20°C and inactivated at 56°C for 30 minutes before being used in the test.

2.4.2. *Saliva.:*

Oro-pharyngeal fluid was collected by the mean of probang from vaccinated groups of sheep for one month post challenge, and from unvaccinated groups of sheep for one year post challenge to show the persistence of the FMDV in pharyngeal region.

2.4.3. *Tissue specimens:*

Tongue epithelia were collected as from calves in contact with unvaccinated groups of sheep after their challenge by one week.

2.5. *Preparation of an inactivated bivalent FMD vaccine:*

FMDV strains (O1/3/93 and A/Egypt/2006) were inactivated by binary ethylene imine 0.001 M and concentrated using polyethylene glycol 6000 (100 g/l liter virus) according to Lei and Scherning-Thiesen (1971), then the vaccine was formulated using Montanide ISA 206 oil and saponine according to Frenkel et al., (1982) and Barnett et al., (1996). FMD virus concentration in the final vaccine formula was adjusted to 10^8 TCID₅₀ per vaccine dose (1 ml).

2.6. *Testing quality of the prepared vaccine:*

The prepared inactivated bivalent FMD vaccine was tested for sterility according to Code of Federal Regulation of USA (1986), while safety for sheep and calves and also potency of the prepared vaccine in guinea pigs were evaluated according to Henderson (1970).

2.7. *Testing efficacy of the prepared vaccine:*

Experimental design:

Experiment (1):

Four groups (G1, G2, G3, G4) of sheep (5 sheep per group) were vaccinated subcutaneously with the prepared bivalent inactivated FMD vaccine (1ml /sheep). Serum samples were collected from vaccinated and non-vaccinated control sheep groups (G5, G6), till challenge time and subjected to SNT for titration of neutralizing FMD antibodies.

Experiment (2):

Sheep in different groups either vaccinated or unvaccinated were exposed to air born challenge by direct contact with infected calves (two calves with each group). Vaccinated sheep groups (G1 and G2) and non-vaccinated control sheep groups (G5, G6) were challenged with type A and type O FMDV for (G1, G5) and (G2, G6), respectively at 21 days post vaccination time. Vaccinated sheep groups (G3 and G4) were challenged with type A and type O FMDV for G3 and G4, respectively at 7 months post vaccination time.

Experiment (3):

Studying role of sheep in transmission of FMDV by putting free unvaccinated calves with challenged unvaccinated sheep groups G5 and G6 (two calves with each group) at one week post challenge and for one week. Serum samples were collected from sheep were subjected for SNT for detection and titration of FMD antibody while oro-pharyngeal fluid samples were collected from calves for FMDV detection using indirect ELISA.

2.5. *Serum neutralization test (SNT):*

It was performed according to Ferreira (1976) by the micro-technique in 96-well micro titer flat bottom tissue culture plates. SNT was carried out using the two strains of FMDV (O and A) at its 8th passage level in BHK cell culture.

2.6. *Indirect Enzyme linked immunosorbent assay (indirect ELISA):*

FMDV antigen was detected in oro-pharyngeal fluid and prepared tissue samples using FMDV antigen detection ELISA kit for serotyping of FMDV O and A, IZSLER: Bresica, Italy (IAH: Pirbright, UK, Lot No.: 01-2011/ 1204269).

3. RESULTS AND DISCUSSION

Table (1): Mean antibody titers of sheep vaccinated with bivalent inactivated FMD vaccine using SNT in experiment 1:

Days Post Vaccination	*Mean SN antibody titers for FMDV			
	Type A		Type O	
	Vaccinated	Control	Vaccinated	Control
0 day	0.18	0.3	0.21	0.3
7 th day	0.94	0.3	1.02	0.3
14 th day	1.30	0.3	1.41	0.3
21 th day	1.66	0.3	1.84	0.3

* Mean Log₁₀ TCID₅₀ serum neutralizing antibody titer.
Protective serum neutralizing antibody titer=1.2.

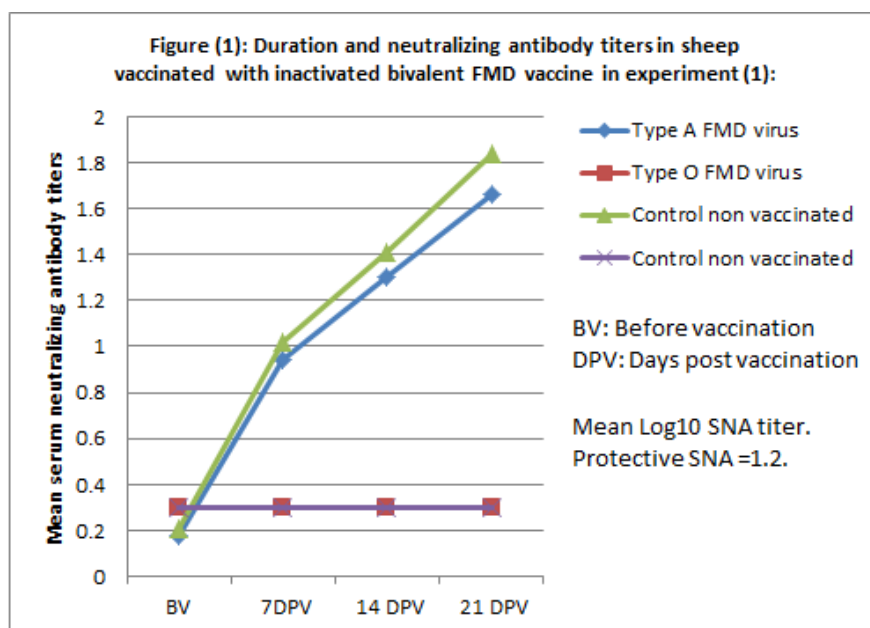


Table (2): Mean antibody titers of sheep vaccinated with bivalent inactivated FMD vaccine using SNT in experiment 2:

Weeks post vaccination	*Mean SN antibody titers for FMDV			
	Type A		Type O	
	Vaccinated	Control	Vaccinated	Control
0	0.22	0.3	0.20	0.3
1	0.91	0.3	0.95	0.3
2	1.32	0.3	1.40	0.3
3	1.68	0.3	1.80	0.3
4	1.96	0.3	2.01	0.3
6	2.53	0.3	2.65	0.3
8	2.53	0.3	2.58	0.3
10	2.22	0.3	2.35	0.3
12	2.16	0.3	2.28	0.3
14	2.05	0.3	2.11	0.3
16	1.94	0.3	2.05	0.3
18	1.87	0.3	1.95	0.3

20	1.75	0.3	1.86	0.3
22	1.63	0.3	1.75	0.3
24	1.56	0.3	1.71	0.3
26	1.47	0.3	1.6	0.3
28	1.42	0.3	1.51	0.3

*Mean Log10 serum neutralizing antibody titer.
Protective serum neutralizing antibody titer=1.2.

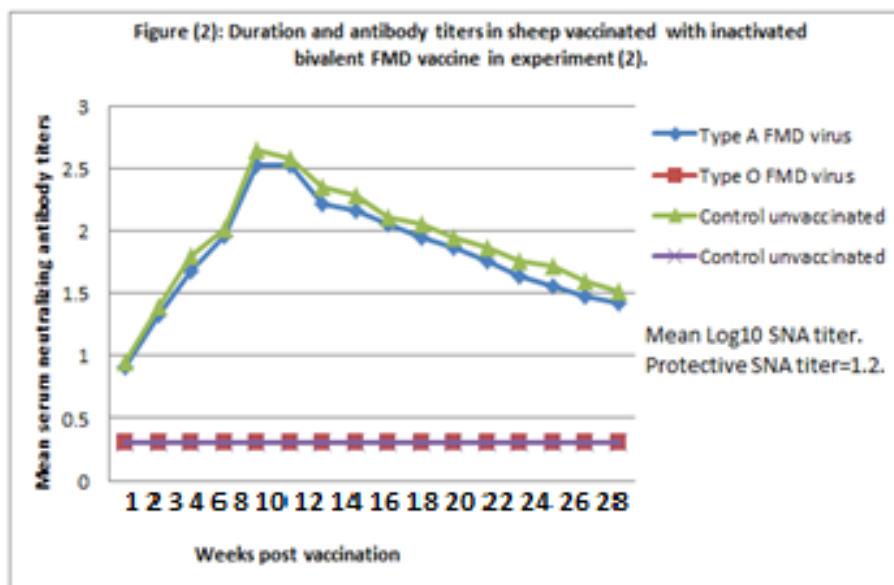


Table (3) Detection of type (A) FMDV in oro-pharyngeal fluid of sheep after challenge using indirect ELISA:

Weeks post challenge	Unvaccinated sheep challenged					Vaccinated sheep challenged 21 days *PV					Vaccinated sheep challenged 7 months PV				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
8	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
10	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
12	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
14	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
16	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
18	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
20	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
22	+	+	+	-	+	*	*	*	*	*	*	*	*	*	*
24	-	+	+	-	+	*	*	*	*	*	*	*	*	*	*
26	-	+	+	-	+	*	*	*	*	*	*	*	*	*	*
28	-	-	+	-	+	*	*	*	*	*	*	*	*	*	*
30	-	-	-	-	+	*	*	*	*	*	*	*	*	*	*
32	-	-	-	-	-	*	*	*	*	*	*	*	*	*	*
34	-	-	-	-	-	*	*	*	*	*	*	*	*	*	*

*PV: Post Vaccination; +=FMDV was detected; -= FMDV was not detected; * = Not done

Table (4) Detection of type (O)FMDV in oro-pharyngeal fluid of sheep after challenge using indirect ELISA:

Weeks post challenge	Unvaccinated sheep challenged					Vaccinated sheep challenged 21 days *PV					Vaccinated sheep challenged 7 months PV				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
8	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
10	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
12	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
14	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
16	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
18	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
20	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
22	+	+	+	+	+	*	*	*	*	*	*	*	*	*	*
24	+	-	+	+	+	*	*	*	*	*	*	*	*	*	*
26	+	-	+	-	+	*	*	*	*	*	*	*	*	*	*
28	+	-	+	-	-	*	*	*	*	*	*	*	*	*	*
30	+	-	+	-	-	*	*	*	*	*	*	*	*	*	*
32	-	-	+	-	-	*	*	*	*	*	*	*	*	*	*
34	-	-	-	-	-	*	*	*	*	*	*	*	*	*	*

*PV: Post Vaccination; +=FMDV was detected; -= FMDV was not detected; * = Not done.

Table (5): Mean antibody titers for types A and O FMDV in sera from unvaccinated sheep after their challenge in experiment 1:

Weeks post Challenge	*Mean SN antibody titers for FMDV	
	Type A	Type O
1	*0.69	0.75
2	1.44	1.77
3	1.98	1.86
4	2.0	2.01
6	2.22	2.19
8	2.31	2.37
10	2.22	2.34
12	2.16	2.34
14	2.1	2.28
16	2.04	2.1
18	1.65	1.86
20	1.47	1.68
22	1.2	1.29
24	0.96	1.09
26	0.75	0.87
28	0.6	0.66
30	0.45	0.54
32	0.27	0.39
34	0.12	0.24
38	0.06	0.12
40	0	0.06
44	0	0
48	0	0

*Mean Log₁₀ serum neutralizing antibody titer.
Protective serum neutralizing antibody titer=1.2.

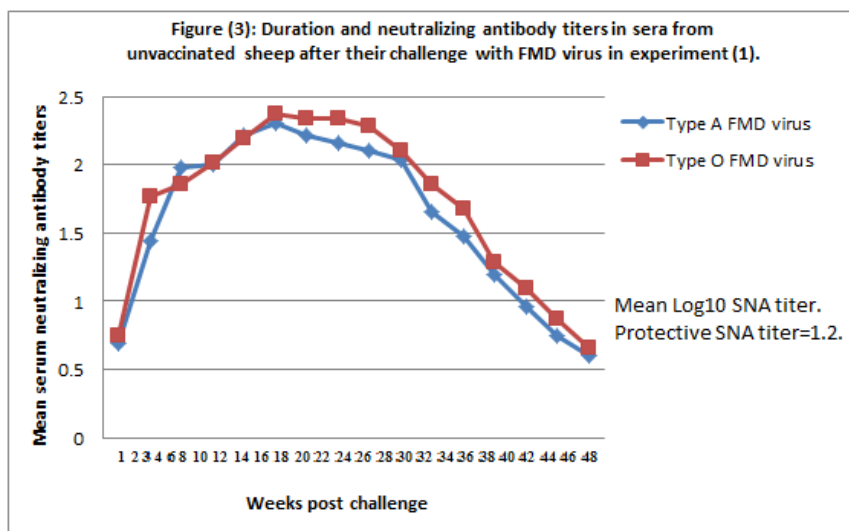


Table (6) Detection of FMDV in tongue epithelia from free calves in contact with challenged unvaccinated sheep using Indirect ELISA.

Animal Group	FMD virus type (A)	FMD virus type (O)
Calves Group (7)	+ve	-ve
Calves Group (8)	-ve	+ve

The prepared inactivated bivalent FMD vaccine (O, A) was ensured for sterility and safety for sheep and calves then potency was evaluated in Guinea pigs that proved to be highly potent as the number of guinea pig protective dose 50% (GPPD₅₀) for type A FMDV equals 33.6/dose and for type O FMDV equals 50.12/dose. This result indicated the high potency of the prepared vaccine which agree with Barnett and Carabin, (2002) who mentioned that highly potent vaccines usually have ≥ 6 protective dose 50% (PD₅₀). Then the prepared vaccine was inoculated in sheep and the humeral immune responses were tested using SNT.

Experiment (1):

The mean serum neutralizing antibody titers started with protective titers at 14th day post vaccination (with 1.30 log₁₀, and 1.41 log₁₀ for FMDV types A and O, respectively), then reached its peak at 21th day post vaccination (1.66 log₁₀ and 1.84 log₁₀ for FMDV types A and O, respectively) in vaccinated sheep groups G1 and G2, respectively as shown in table (1) and figure (1). The mean serum protective neutralizing antibody titers started from 2nd week post vaccination (1.32 log₁₀ and 1.4 log₁₀ for FMDV types A and O, respectively) in vaccinated sheep groups G3 and G4, respectively and persisted in protective level until the end of 7 months post vaccination as shown in table (2) and figure (2). Control non vaccinated sheep (G5 and G6) showed poor antibody titers for both types A and O FMDV. These results showed that the prepared inactivated bivalent FMD vaccine containing saponin as immuno-stimulant was highly potent in sheep for both type A and O of FMDV within both the short period (21 days) and long period (7 months) of vaccination in experiment. These results were not far from results of Ibrahim (2011) who recorded that immune response of sheep to FMD vaccine with saponin and Montanide ISA 206, persisted for 36 weeks post vaccination.

Experiment (2):

Challenge of vaccinated groups of sheep, was done at 21 days post vaccination in sheep groups (1 and 2), and at 7 months post vaccination in sheep groups (3 and 4) revealed no rise in body temperature, absence of clinical signs of FMD on them and negative detection FMDV type A or O using indirect ELISA in oro-pharyngeal fluid

samples collected for one month post challenge time as shown in tables (3) and (4). These results agreed with the results of Barnett et al., (2004) who vaccinated sheep by high potent vaccine (had a PD_{50} value of 41 in cattle) and after challenged them by direct contact with infected pigs, found that there is no FMD virus detected in oro-pharyngeal fluids samples which collected for 42 days after challenge.

Challenge of unvaccinated sheep in groups G5 and G6 showed elevated body temperatures and positive detection of FMDV in oro-pharyngeal fluid samples collected from them (using indirect ELISA) that persisted for 22 - 30 weeks and 24 -32 weeks post challenge time for type A and type O FMDV, respectively as shown in tables (3 and 4). This result that showed the persistence of FMDV in oro-pharyngeal fluid of infected sheep as a carrier state ranged from five to eight months agreed with the study of De Clercq (2002) who found that goats and sheep could harbor FMDV for up to nine months after infection and Doel (2003) who found that carrier state of FMD appears to last up to six to nine months in goats and sheep.

Serum neutralizing antibody titer to FMDV evaluated using SNT showed that the infected non vaccinated sheep have antibody titer in their sera which quickly elevated reach to high titers and then rapidly decreased to lower titers than the vaccinated one, titers raised from the first week post challenge, reached the peak at 8th week post challenge with high titer reach to (2.31log₁₀ and 2.37log₁₀), and then decreased to reach to zero at (40th and 44th) week post challenge for FMDV types A and O, respectively as shown in table (5) and figure (3). This result indicated active virus replication in infected animals that agree with those obtained by De Clercq (2002) who found that sheep had high neutralizing FMD antibody titer for 9 months post infection and El-Sayed et al. (2012) who mentioned that naturally infected calves have high neutralizing FMD antibody titer for up to 36 weeks post infection.

Experiment (3):

To study the role of carrier sheep in transmitting FMD virus, Calves free from infection were put in contact to each group of unvaccinated sheep challenged with FMDV one week post challenge. They showed elevated body temperatures, signs of the disease and positive detection of types A and O FMDV in tongue epithelial tissue samples collected from them (using indirect ELISA) as shown in table (6). This result denoted that sheep play an important role in the transmission of FMDV because of their ability to become carriers and reservoirs for further infection and spread of the disease which agreed with the studies of Barnett and Cox (1999) and Alexandersen et al., (2002) who mentioned that contagiousness of FMDV is reflected by the wide host-range of the virus, the amount of infectivity excreted by affected animals, the low doses required to initiate infection and the many routes of infection.

4- CONCLUSIONS

From the results of our experiment, it could be concluded that sheep contribute to great extent to the persistence or spread of FMDV as unvaccinated sheep plays a role in transmission of FMDV "Silent transmitter" or "carrier state" at convalescence from infection. Highly potent, inactivated FMDV vaccine can confer early and rapid protective immunity against aerosol challenge in sheep within 14 days post vaccination and inhibited local virus replication and prevent the carrier state in sheep.

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