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EFFECT OF VACCINE ON THE ROLE OF SHEEP AS A CARRIER FOR TRANSMISSION OF FOOT AND MOUTH DISEASE VIRUS.

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A B S T R A C T

Sheep plays an important role as a carrier in the persistence and the spread of Foot and Mouth Disease Virus (FMDV). The ability of high potency inactivated bivalent FMDV (A and O) vaccine to inhibit local replication of FMDV and carrier state in sheep was studied. The vaccine induced protective titer of serum neutralizing antibody14th days post vaccination and protected vaccinated sheep at challenge after 21 days or 7 months post vaccination in comparison to non-vaccinated one. Oro-pharyngeal fluid samples collected from vaccinated group of sheep showed absence of FMDV antigen for one month post challenge time using ELISA, conversely to non-vaccinated one which transmitted viral infection to contact calves. Our results reflected the important role of highly potent FMDV vaccine in prevention of the carrier state in sheep.

Key Words: ELISA, FMD, Sheep, SNT, Vaccine

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1. INTRODUCTION

Foot and Mouth Disease (FMD) is a highly contagious viral disease attacking cloven-hoofed animals especially cattle, pigs and sheep [6]. The disease is caused by a member of family Picornaviridae, genus Aphthovirus [18] including seven main serotypes (O, A, C, SAT_1, SAT_2, SAT_3, and Asia), as well as numerous intratypic strains. In Egypt, where the disease is highly endemic, prophylactic vaccination against FMDV (A and O) is the only means of control [1, 9-11]. Animals play an important role in the epidemiology and transmission of FMD being suspected to have frequently sub-clinical from which infection is transmitted. So, such animals may become carriers and thus be a potential source of new outbreaks [16, 21]. So, this experiment was designed to study the role of sheep in transmission of FMD and establish whether a correlation exists between the vaccine potency and the immune response and local viral replication and persistence in sheep.

2. MATERIALS AND METHODS

2.1. *Virus strains:*

FMD virus strains type A/Egypt 2006 and O1/3/93 were locally isolated in Egypt, identified and typed at FMD department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, and was confirmed by FMD world reference laboratory (WRL), Pirbright, UK.

2.2. *Experimental Animals:*

2.2.1. *Sheep:*

Sheep of local breed (n=33), 50-70 kg body weight and 1-3 years old, were divided into 7 groups, 4 groups of which were vaccinated with the prepared FMD vaccine (2 groups of them were challenged

after 21 days and the other 2 groups were challenged 7 months post-vaccination). The remaining 3 groups, two of which were kept as control (unvaccinated) and the last one group was used in safety test.

2.2.1. *Calves:*
Twenty three calves (local breed) of 6-8 months old and 200 – 300 kg body weight were divided into 10 groups (six groups were infected by 10^4 $BTID_{50}$ of virulent homologous virus strain – three groups of them with type A/Egypt2006 FMDV and the other three groups with type O1/3/93 FMDV- two groups were kept with unvaccinated groups of sheep after challenge by one week, one group was used in titration of FMDV used in the challenge, and one group for the safety of the prepared vaccine.

2.3. Samples:
2.3. 1. *Serum samples:*
Sera were collected from vaccinated groups of sheep tell 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 min. before being used in the test.

2.3. 2. *Saliva:*
Oro-pharyngeal fluid was collected by the mean of probing 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.

2.3. 3. *Tissue specimens:*
It was collected from calves in contact with unvaccinated groups of sheep after their challenge by one week.

2.4. *Preparation and testing of vaccine and vaccination of sheep:*
FMDV strains (O1/3/93 and A/Egypt2006) were inactivated by BEI and concentrated using polyethylene glycol 6000 (100 gm/1 liter virus) **[**17], then the vaccine was formulated using Montanide ISA 206 oil and saponine [5, 13]. The FMD virus concentration in the final vaccine formula was adjusted to 10^8 $TCID_{50}$ per vaccine dose (1 ml). After being tested for sterility and safety, the number of $GPPD_{50}$ was calculated for the prepared vaccine, for type A virus equals 33.6/dose and for type O virus equals 50.12/dose. Four groups of sheep were vaccinated subcutaneously with the prepared bivalent inactivated FMD vaccine (1ml /sheep). Serum samples were collected from vaccinated sheep till challenge time for SNT.

2.5. *Challenge of sheep:*
Sheep in different groups either vaccinated or unvaccinated were exposed to air born challenge (groups with FMDV type A and groups with FMDV type O) by direct contact with infected calves. Serum samples from them were subjected for SNT for antibody titration and oro-pharyngeal fluid samples were collected for FMDV detection using ELISA.

2.6. *Studying role of sheep in transmission of FMDV:*
Free unvaccinated calves were put with challenged unvaccinated sheep for one week (group with sheep challenged with FMDV type A and group with sheep challenged with FMDV type O). Tissue specimens were collected from calves after infection for FMDV detection using ELISA.

2.7. *Serum neutralization test (SNT):*
The test was performed by the micro-technique [12] in flat bottom tissue culture microtiter plates. SNT was carried out in 96-well micro titer plate using FMDV two strains as an antigen at its 8th passage level in BHK cell culture. The SN titer was expressed as the $\log_{10}$ of the final serum dilution which protected 50% of wells [20].

2.8. *Indirect sandwich Enzyme linked immunosrobent assay (FMDV Antigen Detection ELISA):*

The FMDV antigen were detected in oro-pharyngeal fluid and prepared tissue samples using ELISA kit (IAH: Pirbright, UK, Lot No.: 01-2011/ 1204269).

3. RESULTS AND DISCUSSION

Foot and Mouth disease (FMD) is one of the most wide spread diseases affecting cloven footed animals with detrimental effects on meat and milk production, rather than convalescent animals became carriers and source of infection [15]. In Egypt, FMD assumes an enzootic form and attacks susceptible animals causing high losses in milk and meat production [19]. Routine prophylactic vaccination has been conducted with a locally produced inactivated bivalent FMD vaccine (O, A).
Sheep is a domestic animal that lives in almost all cattle farms and its role in maintaining and transmitting FMDV cannot be neglected. So the aim of our work is to through the light on the correlation between the vaccine and immune response and prevention of local viral replication and persistence as well as the role of sheep in transmitting FMD.
An inactivated bivalent FMD vaccine (O, A) was prepared, ensured for sterility and safety then potency was evaluated in Guinea pigs using Guinea Pigs Protective Dose 50 ($GPPD_{50}$) calculated [20]. The vaccine proved to be highly potent as the number of $GPPD_{50}$ of the prepared vaccine for type A virus equals 33.6/dose and for type O virus equals 50.12/dose. The prepared vaccine was used for vaccination of sheep and the humeral immune responses were tested using SNT.
In the first and second vaccinated groups of sheep that being challenged after 21 days post vaccination, the protective neutralizing serum antibody titer (1.30 $\log_{10}$ $TCID_{50}$) for FMDV type A and (1.41 $\log_{10}$ TCID50) for FMDV type O were started at 14th days post vaccination, and the mean SNT titers after 21 days post vaccination were (1.66 $\log_{10}$ $TCID_{50}$) for type A and (1.84 $\log_{10}$ $TCID_{50}$) for type O (table1, fig. 1).

Table 1 Mean SNA titers of sheep vaccinated with bivalent inactivated FMD vaccine using SNT:

Days Post Vaccination	*Mean SNA titers	
	FMD virus A/ Egypt 2006	FMD virus O1/3/93
0 day	0.18	0.21
7th day	0.94	1.02
14th day	1.30	1.41
21th day	1.66	1.84

* Mean Log_{10} $TCID_{50}$ serum neutralizing antibody titer. Protective serum neutralizing antibody titer=1.2

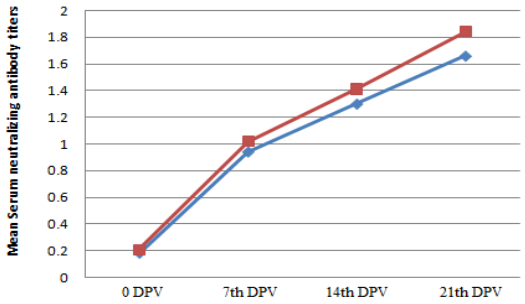

Fig.1 Mean SNA titers of sheep vaccinated with bivalent inactivated FMD vaccine using SNT. (◆) FMDV A/Egypt 2006. (■) FMDV O1/3/93.

In the third and fourth vaccinated groups of sheep that being challenged after 7 months post vaccination the protective neutralizing serum antibody titer for type "A" (1.32 $\log_{10}$ $TCID_{50}$) and type "O"(1.4 $\log_{10}$ $TCID_{50}$) was started from 2nd week post vaccination and persisted in protective level until the end of 7 months (table 2, fig. 2). These results were not far from those who recorded that immune response of vaccinated sheep with saponine + DOE Montanide ISA 206 vaccine persist for 36 weeks post vaccination [14].
Challenge of all groups of vaccinated sheep 21 days (groups 1 and 2) and 7 months (groups 3 and 4) after vaccination and unvaccinated sheep is done by direct contact with calves infected by 10^4 BTID50 of virulent homologous strain (O1/3/93 and A/Egypt/2006), according to the group of sheep. The observation of all groups of vaccinated sheep after challenge revealed no rise in body temperature or clinical signs of FMD appeared on them in

comparison to unvaccinated group showing elevated body temperature.

Oro-pharyngeal fluid samples were collected from individual sheep up to one month post-challenge in all groups of vaccinated and unvaccinated sheep using FMDV antigen detection ELISA.

The result revealed that there is no FMD virus type A or O were detected in isolated sample from groups of vaccinated sheep for one month post challenge time.

Table 2 Mean SNA titers of sheep vaccinated with bivalent inactivated FMD vaccine using SNT

Weeks post vaccination	*Mean SNA titers	
	FMD virus A/ Egypt 2006	FMD virus O1/3/93
1	0.91	0.95
2	1.32	1.40
3	1.68	1.80
4	1.96	2.01
6	2.53	2.65
8	2.53	2.58
10	2.22	2.35
12	2.16	2.28
14	2.05	2.11
16	1.94	2.05
18	1.87	1.95
20	1.75	1.86
22	1.63	1.75
24	1.56	1.71
26	1.47	1.6
28	1.42	1.51

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

FMD virus either type A or type O was detected in samples collected from groups of unvaccinated sheep post challenge time which persisted for 22 - 30 weeks and 24 - 32 weeks for type A and type O, FMD virus respectively (tables 3&4). These results agreed with that vaccinated sheep by high potent vaccine with high antigen payload (had a PD50 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 [4].

The result showed that the persistence of FMD virus in oro-pharyngeal fluid of infected sheep as a carrier state ranged from five to nine months (tables 3&4), and this result agreed with the study which found that goats and sheep could harbor FMDV for up to nine months after infection [7] and that found carrier state of FMD appears to last up to six to nine months in goats and sheep [8].

The role of sheep in transmitting FMD showed that FMD virus type A was detected in samples collected from two calves in contact to unvaccinated sheep challenged indirectly with FMD virus type A. FMD virus type O was also detected in samples collected from two calves in contact to unvaccinated sheep challenged indirectly with FMD virus type O. This result denoted that sheep and goats play an important role in the epizootological nature of FMD because of their ability to become carriers and reservoirs for further infection and spread of the disease which agreed with the studies which 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 [2, 3].

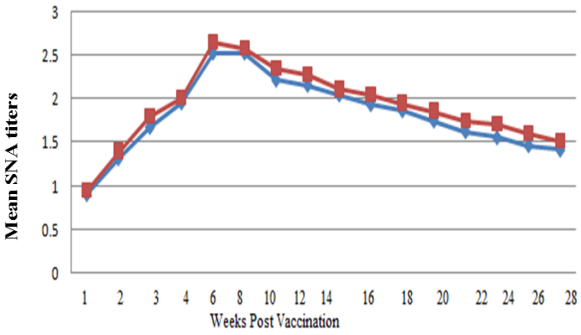

Fig. 2 Mean SNA titers of sheep vaccinated with bivalent inactivated FMD vaccine using SNT. (◆) FMDV A/Egypt 2006. (■) FMDV O1/3/93.

Table 3 Detection of FMD virus type (A) in oro-pharyngeal fluid of sheep post challenge using indirect sandwich ELISA

Weeks Post Challenge	ELISA detection of FMD virus type (A) in oro-pharyngeal fluid.														
	Unvaccinated challenged sheep					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 detected; * = Not done.

Table 4 Detection of FMD virus type (O) in oro-pharyngeal fluid of sheep post challenge using indirect sandwich ELISA

Weeks Post Challenge	ELISA detection of FMD virus type (O) in oro-pharyngeal fluid														
	Unvaccinated challenged sheep					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 detected; * = Not done.

In conclusion, sheep contribute to great extent to the persistence or spread of FMD virus 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 and inhibited local virus replication and prevent the carrier status in sheep.

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مجلة بنها للعلوم الطبية البيطرية
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تأثير التحصين على دور الاغنام فى نقل عدوى فيروس الحمى القلاعية.

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الملخص العربى

تلعب الاغنام دورا مهما كحامل للعدوى فى استمرار وانتشار فيروس مرض الحمى القلاعية. تم دراسة قدرة لقاح الحمى القلاعية المثبط الثنائى العترة (A وO) عالى الفعالية على تثبيط التضاعف المحلى للفيروس ومنع حالة حامل العدوى فى الاغنام. أعطى اللقاح مستوى واقى لعيارية الاجسام التعادلية المضادة عند 14 يوما و أعطى حماية للاغنام المحصنة عند تطبيق اختبار التحدى عليها بعد 21 يوم أو 7شهور بعد التحصين وذلك مقارنة بالاغنام غير المحصنة. أظهر اختبار الاليزا غياب الفيروس فى عينات اللعاب المجمعة من الاغنام المحصنة لمدة شهر بعد تطبيق اختبار التحدى وذلك على عكس الاغنام الغير محصنة التى ظهر فيها الفيروس واستطاعت نقل عدوى الفيروس لعجول سليمة مخالطة لها. أظهرت النتائج الدور المهم للقاح عالى الفعالية لفيروس مرض الحمى القلاعية فى منع حالة حامل العدوى فى الاغنام.

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