Egyptian J. Virol. 5 (1): 260-270 (2008)

The immune response of different farm animals vaccinated with bivalent FMD vaccine

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Cellular and humoral immune responses to bivalent FMD vaccine were estimated in vaccinated cattle, buffaloes, sheep, goats and camels. Five animals of each species were vaccinated using a dose of 1 ml for sheep and goats and 2 ml for cattle, buffaloes and camels inoculated subcutaneously. It was found that both of the immune parameters were generally increased gradually to reach the highest value of cellular immunity by the 28th day, while that of neutralizing antibodies was reached by the 6th week to the 10th week post vaccination. It was noticed that vaccination of cattle and sheep resulted in higher and longer duration of immune response than those obtained in vaccinated goat and buffaloes. While in vaccinated camels there was no evidence of antibodies against FMD. On the other hand, there were no significant differences between the levels of cellular immunity in different vaccinated animal species.

Key words: FMD-vaccine,

INTRODUCTION

Rotavirus is a nonenveloped segmented (11 segments), double-stranded RNA (dsRNA) virus belonging to family Reoviridae. Rotaviruses are the single most important etiological agent of diarrheal disease in infants and young animals throughout the world (Estes, 2001).The rotavirus virion consists of a three concentric protein shells, or layers. The innermost layer, or core, is composed of the VP2 protein, the middle layer is composed of the VP6 protein, and the outer layer is composed of the major surface glycoprotein, VP7 (glycoprotein, G) and haemagglutinin spike, VP4 (Protease sensitive, P); both enclose neutralization antigens **(Estes and Cohen, 1989).**

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(Received March 2008)
(Accepted May 2008)

infection of cattle, pigs, sheep, goats, buffaloes and artiodactyls wildlife species. It is characterized by fever; vesicles in mouth and on the muzzle, teats and feet; and death in young animals (**OIE annual status, 2006**). All species of deer and antelope, elephant and giraffe are susceptible to FMD but camels are resistant to natural infection. Smaller camelids such as alpacas and llamas, although susceptible, are probably of no epidemiological significance. Mice and guinea pigs can be infected experimentally (**Armstrong et al., 1998** and **Moussa et al., 1998**).

Also dromedaries can contract the disease after experimental infection or through close contact with FMD diseased livestock but do not present a risk in transmitting FMD to susceptible animals (**Warnery et al., 2004** and **Shawky et al., 2006**).

It was stated that serotypes O and A of FMD virus has been isolated from sheep and goats (**Kitching et al., 2002** and **Assem, 2006**). In addition, serological surveys revealed an evidence of occurrence of the disease in buffaloes (**Starver et al., 1970; Abu Baker, 1996** and **Abeer et al., 2002**). The disease is considered enzootic in Egypt and many outbreaks have recurrently occurred involving most governorates (**Moussa et al., 1974; Daoud et al., 1988; El-Nakashly et al., 1996** and **Farrag et al., 2004 and 2005**). The causative serotype in such outbreaks was mainly type O but the last outbreak was found to be due the type A of FMD virus (**Abdel-Rahman et al., 2006**).

A new locally inactivated bivalent vaccine was generated containing the two types of the virus. Such vaccine was found to be safe and potent and helps to overcome the challenge and natural infection of animals with the virulent viral strains. Both cellular and humoral immune responses of animals usually share crucial role in the protection against FMD where the first one appears mainly more rapid than the second one but last shorter. Investigation of the immunogenicity of the bivalent FMD vaccine showed that it is a potent and offers a protective immunity in vaccinated animals (**Abd El-Rahman et al., 2007**).

The present work was designed to investigate and evaluate the cellular and humoral immune response to the bivalent FMD vaccine in cattle, buffaloes, sheep, goats and camels.

MATERIAL AND METHODS

1- Animals:

Five male Balady sheep and goats of about 6 months of age, were purchased from El-Wadi El-Gedid, weighing about 50-60 Kgm. Male native breed cattle and buffaloe calves of about 6-9 months of age and male camel of 9 months age, were screened and found to be free from antibodies against FMD using serum neutralization test.

These animals were vaccinated with the local inactivated bivalent FMD vaccine in a dose of 1 ml for sheep and goats, 2 ml for cattle, buffaloes and camel, inoculated subcutaneously. In addition 2 animals of each species were kept without vaccination as control.

Serum samples were collected from all animals every week post vaccination till the 4^{th} week, then every 2 weeks till the 20^{th} week, to estimate the humoral immunity. In addition to heparinized blood samples which were collected from all animals on the 3^{rd}, 7^{th}, 14^{th}, 21^{st}, 28^{th} and 35^{th} days post vaccination to evaluate the cellular immunity.

2- Inactivated bivalent foot and mouth disease vaccine:

It was supplied by the Department of foot and mouth disease vaccine research, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo. It was used for vaccination of experimental animals.

3- FMD virus strains:

BHK cell culture adapted FMD virus serotypes O and A were supplied by the same department and used in SNT.

4- Serum Neutralization test (SNT):

The obtained samples from vaccinated animals were inactivated at 56°C for 30 minutes then subjected to SNT according to **Ferreira (1976).**

5- Lymphocyte proliferation test (L.P.T):

It was carried out as described by **Lucy (1984)** and modified by **Abeer et al. (2002).**

5.1. Phytohaemagglutinin (CPH):

It is none specific mitogen supplied by Biochrom KG, Leo Renstr, 2-6-D-1224, Berlin, Germany.

5.2. Roswell Park Memorial Institute, 1640 medium (RPMI-1640).

5.3. Ficol solution.

5.4. (4, 5 dimethylthiazol-2-y1,2, 5-diphenyltetrazolium bromide (MTT).

5.5.Sodium dodecyle sulphate (SDS):

It were supplied by Sigma Pharmaceutical company.

Results and Discussion

The obtained results of the lymphocyte blastogenesis assay measuring the cell mediated immune response of vaccinated animals showed that the delta optical density (ΔOD) correspond to phytohaemagglutinin (non-specific mitogen) were increased gradually from the 3rd week post vaccination (WPV) to reach its highest value by the 28th day then began to decline by the 35th day. Using FMDV as specific mitogen the ΔOD values were higher than those corresponding to phytohaemagglutinin. This behavior of such immune response was nearly the same among all vaccinated animals but it seems to be of no value in camel when FMDV was used as specific mitogen while use of phytohaemagglutinin as non-specific mitogen reflected in stimulating of cellular immunity.

These findings appear to be in agreement with those of **Halima et al. (1999), Abeer et al. (2002)** and **Abd El-Rahman et al. (2007)** who showed that cellular immunity plays an important role in the immune response to the inactivated foot and mouth disease vaccine. Also the observed findings in vaccinated camels that can't produce antibodies were supported by those of **Shawky et al. (2006)** who reported that camel can contract FMD after experimental infection but do not produce serum antibodies as in the case of vaccination.

On the other side, serum neutralization test revealed that specific FMD neutralizing antibodies were detectable from the 1st week post vaccination and reached the highest level by the 10th WPV in vaccinated cattle and sheep (2.7 $\log_{10}$ – 2.25 $\log_{10}$) and by the 8th WPV in buffaloes and goats (1.95 $\log_{10}$ – 1.95 $\log_{10}$) but in vaccinated camels there were no developing serum neutralizing antibodies. The mean serum neutralizing antibody titers in vaccinated cattle and sheep was still protective till the 16th WPV (1.5 $\log_{10}$ – 1.2 $\log_{10}$) while it was still protective till the 14th WPV in goats (1.2 $\log_{10}$), while in

vaccinated buffaloes the mean serum neutralizing antibody titer still protective till the 12th WPV (1.2 log_{10}).

These findings come parallel to those recorded by **Barteling and Vreeswijk (1991)** and **Abd El-Rahman et al. (2006)** who found that the highest mean titer of FMD antibodies in vaccinated cattle was obtained at 8th – 10th WPV with a duration of immunity up to 16 WPV. Also **Halima et al. (1999)** and **Abeer et al. (2002)** reported that in FMD vaccinated sheep the mean serum neutralizing antibody titer reached the highest level within 10th WPV and still protective up to 16th WPV. **Assem (2006)** and **Fathia (2003)** mentioned that the highest mean FMD serum neutralizing antibody titer was reached at 8th WPV in foot and mouth disease vaccinated goats and was still protective till the 12th to 14th WPV.

In addition **Abu Bakr (1996)** and **Abeer et al. (2002)** found that the duration of humeral immunity induced by foot and mouth disease vaccine in vaccinated buffaloes was 12 WPV, then became within unprotective titer. **Shawky et al. (2006)** reported that experimentally infected and vaccinated camels have no ability to induce antibodies against FMD virus and FMD vaccine.

From the obtained results, it could be concluded that there is a variation in both humeral and cellular immune response to the FMD inactivated bivalent vaccine among various species.

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Table (1) Mean FMD type A serum neutralizing antibody titers (expressed as log_{10}) in different animal species vaccinated with inactivated bivalent FMD vaccine

Animal species	Mean FMD type A antibody titers on week intervals post vaccination												
	Prevac.	1*	2	3	4	6	8	10	12	14	16	18	20
Cattle	0.3	0.6	1.05	1.35	1.65	1.95	2.4	2.7	2.25	1.8	1.5	1.05	0.75
Buffaloe	0.15	0.45	0.9	1.2	1.35	1.65	1.95	1.5	1.2	0.9	0.75	0.45	0.3
Sheep	0.3	0.6	0.9	1.2	1.5	1.65	1.95	2.25	1.95	1.65	1.2	0.9	0.6
Goat	0.3	0.6	0.9	1.2	1.35	1.65	1.95	1.8	1.5	1.2	1.05	0.75	0.3
Camel	0.15	0.3	0.3	0.15	0.15	0.0	0.3	0.15	0.3	0.3	0.0	0.15	0.3

* = weeks post vaccination

Table (2) Mean FMD type O serum neutralizing antibody titers (expressed as log_{10}) in different animal species vaccinated with the inactivated bivalent FMD vaccine

Animal species	Mean FMD type A antibody titers on week intervals post vaccination												
	Prevac.	1*	2	3	4	6	8	10	12	14	16	18	20
Cattle	0.15	0.45	0.9	1.2	1.5	1.8	2.1	2.4	2.1	1.8	1.5	1.5	0.6
Buffaloe	0.15	0.3	0.6	1.2	1.35	1.5	1.8	1.5	1.2	0.75	0.65	0.45	0.3
Sheep	0.15	0.45	0.75	1.2	1.35	1.65	1.95	2.1	1.8	1.5	1.2	0.9	0.45
Goat	0.3	0.6	0.6	1.2	1.35	1.65	1.8	1.65	1.5	1.2	0.9	0.6	0.3
Camel	0.15	0.15	0.3	0.3	0.3	0.15	0.0	0.0	0.3	0.15	0.3	0.3	0.0

* = weeks post vaccination

Table (3) Lymphocyte proliferation test in different animal species vaccinated with inactivated bivalent FMD vaccine

Animal species	Mitogen and used virus	of lymphocyte proliferation / days post vaccination						
		0	3 DPV	7 DPV	14 DPV	21 DPV	28 DPV	35 DPV
Cattle	PHA	0.100	0.200	0.235	0.312	0.380	0.425	0.287
	FMDV	0.117	0.241	0.269	0.341	0.405	0.456	0.315
Buffalo	PHA	0.113	0.194	0.228	0.305	0.365	0.400	0.290
	FMDV	0.120	0.245	0.277	0.334	0.397	0.439	0.311
Sheep	PHA	0.105	0.271	0.242	0.326	0.367	0.431	0.300
	FMDV	0.116	0.261	0.281	0.415	0.412	0.460	0.296
Goat	PHA	0.110	0.205	0.238	0.318	0.371	0.409	0.291
	FMDV	0.114	0.253	0.258	0.420	0.411	0.448	0.309
Camel	PHA	0.109	0.201	0.225	0.275	0.319	0.400	0.290
	FMDV	0.101	0.105	0.109	0.103	0.105	0.100	0.105

Δ OD = delta optical density

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