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Effect of simultaneous vaccination with the bivalent foot and mouth vaccine and bovine ephemeral fever vaccine on the immune response of Cattle

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The present study was aimed to evaluate the vaccination of cattle against bovine ephemeral fever (BEF) and bivalent foot and mouth disease (FMD) strain O and strain A. so fifteen calves, six month old, were vaccinated singly and simultaneously and two calves were left as none vaccinated control. The serum neutralizing antibody titer expressed in log_{10} of FMD (strain O and strain A) in calves vaccinated with bivalent FMD vaccine only were increased from (0.9 log_{10} and 0.9 log_{10}) consequently at the first week post vaccination (WPV) till it reached (2.7 log_{10} and 2.85 log_{10}) for type O and type A respectively by the 8th week. These titers were reduced to (1.2 log_{10} – 1.35 log_{10}) by the 16th WPV. The FMD neutralizing antibody titer in calves vaccinated simultaneously with bivalent FMD and BEF showed increase in the titer from the 1st WPV till reached the highest titer in the 8th WPV (2.55 log_{10} and 2.7 log_{10}) for type O and type A, then decline to (1.2 log_{10} – 1.35 log_{10}) by the 16th WPV. Determination of BEF serum neutralizing antibodies in calves vaccinated with BEF only showed an increase in the titer from (0.15 log_{10}) from 1st week till reached its peak after 8 weeks (1.8 log_{10}), while in calves vaccinated simultaneously with BEF and FMD the serum neutralizing antibody titer reached to 1.85 log_{10} by the 8th week. Evaluation of the cell mediated immunity for both FMD and BEF in vaccinated calves revealed that such immune response was started to increase from the 3rd day post vaccination (DPV) and reached its peak at 21 - 28 (DPV). So, simultaneous vaccination of calves with the bivalent FMD and BEF could be considered of applicable benefit.

Key words:

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INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious disease primarily affecting cloven footed animals and is characterized by vesicular lesions and subsequently by erosions of the epithelium of mouth, nostrils, feet, teats, udder and rumen pillars (4 and 10). The disease considered enzootic in Egypt and many outbreaks have recurrently occurred involving most governorates (12, 14 and 2). The causative serotype of FMD in previous outbreaks was mainly type O but the last outbreak was found to be due to the type A of FMD virus (1). It was well known that vaccination is the basic step and corner stone in controlling FMD as other viral infectious disease (7). The used FMD vaccine in Egypt was the cell culture inactivated vaccine prepared from the local strain O1/3/93 which was used for vaccination of cattle, buffaloes and sheep for longtime. Nowadays and after the isolation of FMD virus type A (A/Egy1/2006) through the importation of live animals from endemic areas (13), a new locally inactivated bivalent vaccine was generated containing the 2 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.

Bovine Ephemeral Fever (BEF) is an infectious disease of cattle characterized by inflammation of mesodermal tissues and enlargement of peripheral lymph nodes (26). BEF is caused by a type species of the genus ephemro virus in family Rhabdovirus (17). The BEF is also controlled by vaccination (9).

Both cellular and humoral immune response of animals usually share crucial role in the protection process against infection where the first one appears mainly more rapid than the second one but last shorter (25).

The purpose of this study is to investigate the effect of simultaneous vaccination of the bivalent FMD and BEF vaccines on the immune response of cattle.

MATERIALS AND METHODS

Animals:

Seventeen cross breed calves of about 6-months old, were screened using serum neutralization test and found to be free from FMD and BEF neutralizing antibodies. The

animals were divided into three groups:

* Group (1): five calves were vaccinated simultaneously with FMD and BEF vaccine and boostered with BEF vaccine 2 weeks post-preliminary vaccination.
* Group (2): five calves were vaccinated with FMD vaccine only.
* Group (3): five calves were vaccinated with BEF vaccine only and boostered 2 weeks post-preliminary vaccination.
* Two calves were left without vaccination as test control.

1- Vaccines:

Bivalent inactivated FMD vaccine and inactivated BEF vaccine were supplied by the Departments of FMD vaccine Research and Pet Animals Vaccines Research, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo.

2- Sera collection:

Jugular vein blood were collected from all vaccinated calves at 0, 1st, 2nd, 3rd, 4th, 6th,8th, 10th, 12th, 14th, 16th week post vaccination (WPV). Sera were separated for determination of antibody titers by SNT.

3- Heparinized blood:

Heparinized blood were collected from all vaccinated calves at 0, 3rd, 7th, 14th, 21st, 28th days post vaccination (DPV). These samples were used for evaluation of cellular immunity by lymphocyte proliferation test (LPT).

4- Mitogen:

5.1 Concavallin-A: it was supplied by Biochromk-1224, Berlin, Germany and used for the invitro lymphocyte blastogenesis assay. According to the manufacturer's direction, cancavallin-A was diluted with RPMI-1640 complete medium.

5.2. Phytohaemagglutinin (PHA): it was supplied by Biochrom KG, Leo Renstr, 2-6-D-1224, Berlin, Germany. It was used in lymphocyte blastogenic assay after its dilution in Roswel Park Memorial Institute (RPMI 1640) complete medium according to manufacturer directions.

1-Roswell Park Memorial Institute, 1640 Medium (RPMI 1640):

RPMI-1640 without sodium bicarbonate was supplied by Sigma Pharmaceutical Company. It was prepared according to manufacturer directions and used for lymphocyte transformation test.

1- Ficol solution:

It was supplied by Sigma Company in a liquid form of a density of 1.077 gm consisted of 57 gm ficol 400 and 9 gm diatizoote dissolved in 100 ml distilled water.

2- 4,5 dimethyl thiazol-2-y1, 2, 5-diphenyltetrazolium bromide (MTT):

MTT was supplied by Sigma Chemical Company and used to estimate the activity of various dehydrogenase enzyme inactive mitochondria of activated lymphocytes.

1. Sodium dodecyle sulphate (SDS):

It was supplied by Sigma Company and used in the lymphocyte transformation test.

2- Tests used to evaluate the humoral and cell mediated immunity in experimental animals:

10.1) Serum neutralization test: the obtained serum samples from vaccinated calves were inactivated at 56 °C for 30minutes then subjected to SNT according to (16). In this test 100-200 $TCID_{50}$ for both FMD and BEF viruses using BHK_{21} cell line.

Lymphocyte proliferation test (lymphocyte blastogenesis assay): it was carried out as (18 and 19) and modified by (20).

RESULTS AND DISCUSSION

Vaccination against viral diseases is important in their control. The aim of the simultaneous vaccination is to reduce the stress on vaccinated animals, help in production of different antibodies which help in protection of vaccinated animals against more than one disease at the same time and safe time and cost.

Foot and mouth disease (FMD) and bovine ephemeral fever (BEF) are viral diseases which decrease the animal productivity. Both diseases are infecting cattle and play a basic role in drastic reduction in meat and milk production.

Table (1) showed the titers of FMD serum neutralizing antibodies, determined in calves vaccinated with FMD only (group 2) and simultaneously with BEF and bivalent FMD vaccines (group 1). In animals vaccinated with FMD vaccine only the antibody titer was starting to increase from the 1st WPV, it was (0.9 log_{10} - 0.9 log_{10}) for FMD virus type O and A respectively, till reached its highest level (2.7 log_{10} -2.85 log_{10}) at the 8th WPV. While in calves simultaneously vaccinated with bivalent FMD and BEF vaccines the mean antibody titers started to increase since 1st WPV and reached its highest level (2.55 log_{10} -2.7 log_{10}) at the 8th WPV for the two types (O and A).

Table (2) tabulated the results of BEF serum neutralizing antibodies in calves vaccinated with BEF vaccine only and boostered 2 weeks post preliminary vaccination (group 3). The antibody titer was 1.35 log_{10} at 3rd WPV. The highest titer was 1.8 log_{10} at the 5th WPV, the titers were protective since 3rd WPV till 16th WPV. The results also showed the titers in case of calves vaccinated simultaneously with both FMD and BEF vaccines and boostered 2 weeks post preliminary vaccination (group 1) where the titer reached 1.85 log_{10} at the 8th WPV.

Tables (3 and 4) showed the evaluation of cellular immune response against both bivalent FMD and BEF vaccines, vaccinated calves with bivalent FMD vaccine only, bivalent FMD and BEF vaccines simultaneously and BEF vaccine only.

The cellular immunity was evaluated by the application of lymphocyte transformation test. On the use of concavallin A and phytohaemagglutinin as none specific mitogen the mean obtained value of delta optical density (ΔOD) start to increase at 3rd day post vaccination and increased gradually to reach its highest value at 21-28 days post vaccination.

It was found that the use of FMDV and BEFV as specific mitogens, resulted in ΔOD values of highest levels in comparison with those obtained with other mitogens in agreement with the finding reported by (22). Similar results obtained by (25, 11, 23, 3 and 21) who stated that cell mediated immunity is a constituent of the immune response against FMDV and BEFV.

The results explore that the simultaneous vaccination of bivalent FMD and BEF vaccines

did not interfere with immune response of vaccine against FMD, and these results were in agreement with (15 and 8) who observed that the cattle vaccinated with FMD and rabies vaccines developed antibodies to each virus as in case the individual vaccine, this example is true due to that rabies and BEF are of the same viral family (Rhabdoviridae), also it was found that the antibodies after vaccination with FMD and rabies not differ from that after vaccination with FMD alone (22).

On the other hand the determination of BEF SN antibodies revealed a titer ranged between 0.4 and 2.11 at 1st and 6th WPV, respectively. In calves received BEF vaccine only while it was ranged between 0.6 and 2.0 at 1st and 6th WPV in calves simultaneously vaccinated by bivalent FMD and BEF vaccines and boostered with BEF vaccine 2 weeks post preliminary vaccination. The cellular immunity of all vaccinated calves with bivalent FMD only, BEF only and simultaneously vaccinated with bivalent FMD and BEF, was measured through the in vitro lymphocyte stimulation with FMD viruses (O and A) and BEF virus as specific mitogen and phytohaemagglutinin as none specific mitogen. The delta optical density (ΔOD) was found to increase gradually from the 3rd day post vaccination to reach its peak by the 4th week then begin to decrease (26 and 27).

So the results of this study indicated that there is no adverse reaction to any of injected vaccines. There is no significant difference in the determination of humoral immunity by different techniques used (SNT and lymphocyte proliferation test).

Table (1) mean FMD neutralizing antibody titer in sera of vaccinated calves with bivalent FMD

Animal groups	Weeks post vaccination																					
	0		1		2		3		4		6		8		10		12		14		16	
	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O
Group (2)	0.3	0.15	0.9	0.9	1.2	1.35	1.6	1.65	2.4	2.25	2.7	2.55	2.85	2.7	2.4	2.1	1.95	1.8	1.65	1.5	1.35	1.2
Group (1)	0.15	0.15	1.05	0.9	1.2	1.2	1.65	1.65	2.25	2.25	2.55	2.4	2.7	2.55	2.4	2.1	1.95	1.8	1.65	1.65	1.35	1.2

Group (2) vaccinated with inactivated FMD vaccine only.
Group (1) simultaneously vaccinated with inactivated bivalent FMD and inactivated BEF vaccines.

Table (2) mean BEF neutralizing antibody titer in sera of vaccinated calves with inactivated BEF.

Animal groups	Weeks post vaccination										
	0	1 .	2	3	4	6	8	.10	12	14	16
Group (3)	0.0	0.15	1.05	1.35	1.65	1.75	1.8	1.8	1.8	1.8	1.8
Group (1)	0.0	0.6	1.05	1.2	1.5	1.8	1.85	1.85	1.85	1.85	1.85

Group (3) vaccinated with BEF vaccine only boostered with BEF vaccine 2 WPV.
Group (1) simultaneously vaccinated with inactivated FMD and BEF vaccines and boostered with BEF vaccine 2 WPV.

Table (3) lymphocyte blastogenesis in calves vaccinated with inactivated bivalent FMD vaccines

Animal groups	Mitogen and used virus	Lymphocyte blastogenesis as measured by optical density (ΔOD)/days post vaccination										
		0	3 DPV *		7 DPV		14 DPV		21 DPV		28 DPV	
			A	O	A	O	A	O	A	O	A	O
Group (2)	PHA **	0.100	0.205	0.210	0.235	0.230	0.321	0.318	0.371	0.365	0.390	0.387
	Conca A ***	0.098	0.200	0.210	0.234	0.230	0.319	0.315	0.370	0.360	0.390	0.385
	FMDV	0.111	0.230	0.241	0.264	0.260	0.370	0.363	0.410	0.401	0.450	0.441
Group (1)	PHA **	0.101	0.200	0.202	0.230	0.228	0.318	0.315	0.359	0.361	0.393	0.381
	Conca A ***	0.105	0.200	0.200	0.231	0.225	0.318	0.311	0.358	0.365	0.390	0.380
	FMDV	0.116	0.233	0.230	0.261	0.258	0.351	0.359	0.400	0.400	0.451	0.450

Group (2) vaccinated with inactivated bivalent FMD vaccine only.
Group (1) simultaneously vaccinated with inactivated bivalent FMD and inactivated BEF vaccines.
* DPV: days post vaccination
** PHA: phytohaemagglutinin
*** Conca A: concavallin A
FMDV : Foot and Mouth Disease virus.

Table (4) lymphocyte blastogenesis in calves vaccinated with inactivated BEF vaccines

Animal groups	Mitogen and used virus	Lymphocyte blastogenesis as measured by optical density (ΔOD)/days post vaccination									
		0	3 DPV *		7 DPV		14 DPV		21 DPV		28 DPV	
			A	O	A	O	A	O	A	O	A	O
Group (3)	PHA **	0.105	0.161	0.158	0.218	0.215	0.310	0.307	.360	0.354	0.300	0.285
	Conca A ***	0.102	0.158	0.153	0.218	0.218	0.308	0.301	0.360	0.358	0.300	0.284
	BEF V	0.113	0.175	0.170	0.245	0.241	0.357	0.352	0.400	0.389	0.325	0.321
Group (1)	PHA **	0.101	0.158	0.154	0.215	0.213	0.307	0.301	0.351	0.350	0.296	0.287
	Conca A ***	0.098	0.158	0.157	0.218	0.215	0.307	0.300	0.354	0.350	0.291	0.289
	BEF V	0.105	0.170	0.168	0.241	0.238	0.360	0.358	0.389	0.387	0.315	0.317

Group (3) vaccinated with a dose of 2 ml of inactivated aluminum hydroxide adjuvanted BEF vaccine.
Group (1) simultaneously vaccinated with inactivated bivalent FMD and inactivated BEF vaccines.
* DPV: days post vaccination
** PHA: phytohaemagglutinin
*** Conca A: concavallin A
BEF V : Bovine ephemeral fever virus.

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