

Effect Of Bovine Ephemeral Fever (BEF) And Sheep Pox Vaccines On The Immune Status Of Foot And Mouth Disease (O, A, SAT2) Vaccinated Calves

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ABSTRACT

In this study, the vaccination of cattle with inactivated bovine ephemeral fever (BEF), live attenuated sheep pox (SP) and inactivated trivalent foot and mouth disease (FMD) vaccines was studied as single and simultaneous application. Twenty one calves were divided into seven groups (three animals each one). The cellular immune response was monitored by MTT based lymphocyte proliferation in peripheral blood mononuclear cell cultures, where it was found that the cellular immune response to FMDV did not affected when simultaneously administered with BEFV, but when administered with SPV only, the cell mediated immune response was earlier with higher levels. On the other hand, there is no significant difference in the determination of antibody levels as measured by (SNT and ELISA) between animal groups vaccinated with FMD and BEF vaccines; while, significant elevation in antibody levels was recorded in the case of vaccination with SP, BEF and FMD vaccines at the same time. So, it can be concluded that simultaneous vaccination of calves with SP, BEF and FMD trivalent vaccines could be considered of applicable benefit.

INTRODUCTION

Foot and Mouth Disease (FMD) is a highly contagious viral disease of high morbidity rate with low mortality except in newly born animals where the mortality rate may reach 50% due to myocarditis (1). FMD serotypes "O" (2), "A" (3) and SAT2" were reported in the years 2012 (4). Eradication and vaccination program make it is a disease that in most countries would like to eradicate (5).

Bovine ephemeral fever (BEF) is an acute non-contagious arthropod-born viral disease of cattle and water buffaloes. BEF is suddenly occurring epidemic disease, which swept through a herd causing fever, prostration, stiffness, lameness and occasional death (6). Control of BEF in Egypt is based on control of vectors and vaccination programs for susceptible host as recommended by (7, 8).

Lumpy skin disease (LSD) is a viral disease, it caused by LSD virus in genus capripox virus of the family Poxviridae that affects cattle (9) and it is closely related antigenically to sheep and goat poxviruses (10).

It produces significant morbidity in cattle (11). All calf breeds, ages and sexes are affected. The disease is more severe in young cows in the peak of lactation period (12). The LSD causes severe production losses throughout the cattle industry (13). LSD is endemic in parts of Africa, where outbreaks may be wide spread.

Since 1989 and 1990, the sheep pox vaccine was used to control LSD outbreaks in Egypt (14, 15). It is widely agreed that vaccination is the only effective method to control the spread of FMD, BEF and LSD viruses infections in endemic countries (16).

Owing to the increase number of vaccines which are administrated to the animals at different ages and times; it is necessary to study the effect of BEF and sheep pox vaccines on the immune response of calves vaccinated with trivalent FMD vaccine, the thing which is aimed by the present study.

MATERIAL AND METHODS

Animals

Twenty one apparently health cross-breed calves about one year old their sera were examined and proved to be susceptible to FMD, SPV and BEF as screened by SNT. The animals were divided into seven groups (three calves/group) as follows:

Group (1): Vaccinated with trivalent FMDV only.

Group (2): Vaccinated with trivalent FMDV and BEFV.

Group (3): Vaccinated with trivalent FMDV and SPV.

Group (4): Vaccinated with trivalent FMDV, SPV and BEFV at the same time.

Group (5): Vaccinated with SPV only.

Group (6): Vaccinated with BEFV only.

Group (7): Kept as non-vaccinated control.

Vaccines

Inactivated trivalent FMD vaccine

Inactivated trivalent FMD vaccine is locally prepared in FMD Research Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo (VSVRI). It is containing types A, O and SAT2 was locally prepared and adjuvanted with ISA-206 according to the method described by (17, 4).

BEF vaccine

Binary ethyleneimine BIE inactivated and aluminium hydroxide gel adjuvanted vaccine. The vaccine was prepared according to (18), in Department of Pet Animal Vaccines Research, VSVRI. The vaccine was used in a dose of 2ml/calf inoculated S/C and boosted after 3 weeks with another 2 ml.

Attenuated sheep pox vaccine (Romanian strain)

Sheep pox live attenuated virus vaccine in Vero cells was supplied by Pox Vaccines Department, VSVRI at a titre of $10^{5.5}$ TCID₅₀/ml. The vaccine was prepared according to (14). Animals were inoculated S/C in tail fold with one field dose of the vaccine 1ml/calf.

Viruses

FMD virus

BHK-21 cell culture adapted FMD virus, serotypes "O" Pan Asia, "A" Iran O5 and "SAT2"/Egy/2012 (4). The titre of the three types was expressed in log₁₀ TCID₅₀ according to (19). Virus strain was used in MTT and serological tests as antigen.

Lumpy skin disease virus (LSDV)

Madin Darby Bovine Kidney (MDBK) tissue culture adapted LSD virus (Ismailia strain) with titre of $10^{6.5}$ TCID₅₀/ml was used in MTT and serological tests as antigen (20).

BEF virus

BEF virus adapted on BHK cell culture according to (18) with a titre of 10^7 TCID₅₀/ml was used for estimation of BEF antibodies in vaccinated animals using SNT.

Heparinized blood

Blood samples were collected on heparin from all calves at 0, 3rd, 7th, 10th, 14th, 21st, 28th, 35th and 42nd days used for evaluation of cellular immunity.

Serum collection

Serum samples were taken from blood samples collected from all calves weekly post vaccination till the 4th week, then every month till the 10th month post vaccination for monitoring of the induced humoral immunity.

Cell culture

MDBK cells

Madin Darby Bovine Kidney line was obtained from Pox Vaccines Department, VSVRI. It was used for SNT with LSD virus.

BHK-21 cells

Baby hamster kidney cell line was obtained from FMD Department, VSVRI. It was used for SNT with FMD virus and BEF viruses.

Tests used for evaluation of immune response

Lymphocyte blastogenesis assay

It was carried out according to (21, 22), for evaluation of the cell mediated immunity.

Serum neutralization test (SNT)

It was carried out using the microtitre technique as described by (23-25).

Solid phase ELISA

It was applied according to (26-28). SNT and ELISA were used for evaluation of humoral immunity.

RESULTS AND DISCUSSION

Ruminants constitute a significant integer of the national animal wealth. Infectious diseases are constraints to the development of improved livestock production to which the solution must be prevention by means of effective immunization program and eradication of vectors (29).

FMD, BEF and LSD are three diseases that cause high economic losses on milk and meat production and herd quality. Control of these diseases is conducted by vaccination (11).

The present work depends on evaluation of cellular and humoral immune response of calves vaccinated with FMDV, BEFV and SPV either simultaneously or in single form.

The cellular immune response was evaluated using lymphocyte transformation test and its results were shown in Table (1) for all vaccinated and control negative calves. The obtained values of mean delta optical density (ΔOD) started to increase at the 3rd day post vaccination (DPV) and reached its highest value at 14 DPV for SPV in group (5) and this result is in agreement with what reported by (20, 30) who reported that SPV can induce immune response in cattle as the same in sheep and stimulation and blast formation of lymphocyte appear from the 3rd day till the 14th DPV.

It was noticed that SPV affected by the presence of FMDV and BEFV inactivated and adjuvant at the time of inoculation showing an increase of the immune system stimulation

resulting in higher cellular immune response against this inoculated vaccine.

ΔOD reached its highest values for FMDV at the 28th DPV in groups (3 and 4) which the calves vaccinated with FMDV and SPV and FMDV, BEFV and SPV, respectively. Similar results were obtained by (31) who compared the immune response to FMDV administered separately or with other cattle vaccines without interference between the immune response of animals for FMDV and other vaccines. The cell mediated immune response of vaccinated calves to FMDV appear to be more affected by the presence of SPV as showing higher in development in groups (3 and 4). These results explore that the SPV act as immunostimulant and increase the cellular immune response against FMDV. These results agreed with (20) who recorded that inoculation of SPV mixed with other vaccines increase the cellular immune response than that against vaccines inoculated solely.

Groups 2, 4 and 6 showed evaluation of cellular immune response to BEFV and FMDV, BEFV, FMDV and SPV and BEF. The ΔOD appeared at 3rd DPV and increased gradually to reach its highest value at the 21-28 DPV. Similar results were obtained by (16, 32) who stated that cell mediated immune response is a constituent of the immune against FMDV and BEFV. The results explore that the vaccination of FMDV and BEFV at the same time did not interfere with immune response to any of them and these results were in agreement with (7) who observed that the cattle vaccinated with FMD and rabies vaccine developed antibodies to each virus as in case the individual vaccine. This example is because rabies and BEF are from same viral family (Rhabdoviridae).

The humoral immune response of all calves vaccinated with trivalent FMDV in different groups were evaluated by SNT and ELISA as shown in tables (2 and 3). Tables (2 and 3) indicated that the developed antibody titres against FMDV (strain O, A, SAT2) in single or at the same time with BEFV were approximately similar to each other. Similar findings were recorded by (18, 33) who found that the vaccination of cattle with FMD and

BEF vaccines at the same time did not affect the magnitude or duration of the immune response when compared with FMDV alone. The circulating antibody titre against trivalent FMDV reached the highest level in vaccinated animals in the 8th week in group (1) and by the 4th week in groups and 2nd week in groups (2, 3 and 4) and still protective till the end of the experiment. These results are supported by (4) who reported that inactivated trivalent oil adjuvant FMD vaccine induce the highest level of antibodies with the 8th week post vaccination.

Also these results were in agreement with (18, 33) who found that cattle vaccinated with FMDV and BEFV developed antibodies to FMD virus as in case of individual vaccination.

SNT and ELISA tests results indicated that in group (3) vaccinated with FMDV and SPV and group (4) vaccinated with FMDV, BEF and SPV afford the higher mean serum neutralizing antibody titres than that in group (1) vaccinated with trivalent FMDV or group (2) which

vaccinated with trivalent FMDV and BEFV. These results agree with that recorded by (34, 35) who mentioned that pox viruses induce para-immunity by activation of phagocytosis stimulate lymphocytes *in-vitro* and *in-vivo* and induce formation and release of endogenous interferon.

Also, these results agree to those obtained by (36-38) who used sheep pox vaccine with other viral vaccines. (20, 39) vaccinated animals simultaneously with FMDV and SPV, and found that sheep pox has non specific immunostimulant effect on immune response against FMDV.

From the obtained results, it could be concluded that cellular and humoral immunity in cattle to the FMD inactivated trivalent vaccine when used in animals vaccinated together with SPV and BEF at the same time induce better immune response than those vaccinated with FMDV alone or together with BEFV only.

Table 1. Cell mediated immune response in cattle groups expressed by (Δ OD)

Animal groups	Used mitogen	Δ OD / Days Post Vaccination							
		0	3	7	14	21	28	35	42
Group 1	PHA	0.101	0.193	0.234	0.321	0.380	0.398	0.311	0.247
	FMDV	0.098	0.230	0.281	0.368	0.400	0.472	0.340	0.284
Group 2	PHA	0.107	0.200	0.228	0.311	0.367	0.400	0.308	0.230
	FMDV	0.112	0.225	0.276	0.343	0.395	0.461	0.334	0.272
Group 3	PHA	0.114	0.257	0.291	0.369	0.428	0.456	0.375	0.316
	FMDV	0.107	0.300	0.337	0.391	0.442	0.542	0.421	0.356
Group 4	PHA	0.944	0.261	0.287	0.390	0.438	0.460	0.372	0.320
	FMDV	0.112	0.303	0.340	0.400	0.446	0.509	0.416	0.361
Group 5	PHA	0.120	0.195	0.248	0.353	0.332	0.315	0.302	0.250
	SPV	0.108	0.255	0.352	0.402	0.394	0.360	0.335	0.300
Group 6	PHA	0.125	0.190	0.227	0.330	0.375	0.352	0.288	0.234
	BEFV	0.114	0.226	0.279	0.374	0.414	0.392	0.325	0.275
Group 7	PHA	0.063	0.058	0.061	0.060	0.051	0.058	0.065	0.060
	FMDV	0.049	0.040	0.044	0.050	0.055	0.046	0.043	0.530

Group (1): Calves vaccinated with FMDV trivalent vaccine.

Group (2): Calves vaccinated with FMD trivalent vaccine and BEFV vaccine.

Group (3): Calves vaccinated with FMD trivalent vaccine and SPV vaccine.

Group (4): Calves vaccinated with FMD trivalent vaccine, BEFV vaccine and SPV vaccine.

Group (5): Calves vaccinated only with SPV vaccine.

Group (6): Calves vaccinated only with BEFV vaccine.

Group (7): Control unvaccinated calves.

PHA: Phytohaemagglutinin.

Table 2. Mean serum neutralizing test in different vaccinated cattle groups using SNT

Time Post Vaccination	Groups												G5	G6	G7
	G1			G2			G3			G4					
	A	O	SAT2	A	O	SAT2	A	O	SAT2	A	O	SAT2			
0	0.3	0.0	0.45	0.0	0.15	0.3	0.6	0.45	0.15	0.3	0.45	0.0	0.6	0.6	0.15
1 WPV	0.9	0.6	1.05	0.6	0.75	0.9	1.2	1.05	1.05	1.05	1.2	0.9	0.9	1.05	0.3
2 WPV	1.2	1.05	1.2	1.05	1.2	1.35	1.8	1.65	1.5	1.5	1.8	1.65	1.2	1.2	0.3
3 WPV	1.5	1.35	1.5	1.2	1.5	1.65	2.25	2.1	2.1	2.1	2.25	1.95	1.5	1.5	0.15
4 WPV	2.1	1.95	1.8	1.8	2.1	2.25	2.7	2.4	2.55	2.55	2.4	2.1	1.8	1.8	0.3
2 MPV	2.7	2.55	2.25	2.4	2.55	2.7	2.55	2.55	2.7	2.7	2.7	2.4	1.8	2.25	0.45
3 MPV	2.7	2.4	2.4	2.4	2.55	2.4	2.4	2.25	2.55	2.4	2.55	2.25	2.1	2.4	0.3
4 MPV	2.55	2.25	2.1	2.1	2.4	2.25	2.25	2.1	2.4	2.25	2.4	2.1	2.1	2.1	0.45
5 MPV	2.4	2.1	1.95	1.95	2.25	2.1	2.1	1.95	2.25	2.1	2.25	1.95	1.8	1.65	0.15
6 MPV	2.25	1.95	1.8	1.8	2.1	1.95	1.95	1.8	2.1	1.95	2.1	1.8	1.5	1.65	0.15
7 MPV	2.1	1.8	1.65	1.65	1.95	1.8	1.8	1.65	1.95	1.8	1.95	1.65	1.5	1.5	0.3
8 MPV	1.95	1.65	1.5	1.5	1.8	1.5	1.65	1.5	1.8	1.5	1.8	1.5	1.2	1.35	0.3
9 MPV	1.8	1.5	1.35	1.2	1.65	1.35	1.5	1.35	1.65	1.35	1.65	1.35	1.2	1.2	0.45
10 MPV	1.5	1.35	0.9	0.9	1.35	1.2	1.35	1.05	1.35	1.2	1.35	1.2	0.9	1.05	0.45

N.B. Neutralizing Index (NI) > 1.5 is considered positive.

WPV: Weeks Post Vaccination

MPV: Months Post Vaccination.

Group (1): Calves vaccinated with FMDV trivalent vaccine.

Group (2): Calves vaccinated with FMD trivalent vaccine and posted with BEFV vaccine 4 WPV.

Group (3): Calves vaccinated with FMD trivalent vaccine and SPV vaccine.

Group (4): Calves vaccinated with FMD trivalent vaccine, BEFV vaccine and SPV vaccine and posted with BEFV 4 WPV.

Group (5): Calves vaccinated only with SPV vaccine.

Group (6): Calves vaccinated only with BEFV vaccine and posted with BEFV vaccine 4 WPV.

Group (7): Control unvaccinated calves.

Table 3. Results of ELISA in different vaccinated cattle groups

Time Post Vaccination	Groups												G5	G6	G7
	G1			G2			G3			G4					
	A	O	SAT2	A	O	SAT2	A	O	SAT2	A	O	SAT2			
0	0.6	0.45	0.75	0.0	0.45	0.75	0.9	0.75	0.65	0.6	0.75	0.6	0.9	0.85	0.45
1 WPV	1.05	0.95	1.25	0.75	0.95	1.05	1.45	1.25	1.35	1.35	1.5	1.1	1.2	1.35	0.6
2 WPV	1.5	1.2	1.4	1.35	1.55	1.55	2.0	1.95	1.85	1.85	2.14	1.95	1.55	1.5	0.3
3 WPV	1.95	1.65	1.73	1.55	1.84	1.85	2.45	2.4	2.35	2.3	2.55	2.15	1.85	1.95	0.45
4 WPV	2.3	2.1	2.15	2.15	2.45	2.55	3.0	2.8	2.85	2.85	2.75	2.5	2.0	2.1	0.6
2 MPV	2.85	2.8	2.6	2.75	2.85	3.0	2.85	2.8	2.95	3.1	2.95	2.7	2.0	2.55	0.55
3 MPV	2.9	2.75	2.8	2.7	2.9	2.7	2.7	2.65	2.8	2.72	2.85	2.65	2.5	2.75	0.6
4 MPV	2.75	2.5	2.45	2.4	2.75	2.55	2.55	2.4	2.65	2.55	2.65	2.45	2.5	2.3	0.55
5 MPV	2.65	2.45	2.25	2.2	2.6	2.4	2.38	2.25	2.45	2.45	2.55	2.25	2.1	1.95	0.45
6 MPV	2.4	2.35	2.15	2.0	2.4	2.2	2.25	2.1	2.3	2.25	2.3	2.1	1.8	1.95	0.45
7 MPV	2.35	2.2	2.0	1.95	2.15	2.0	2.1	2.0	2.1	2.0	2.1	1.95	1.8	1.85	0.6
8 MPV	2.1	2.0	1.95	1.8	2.0	1.85	1.95	1.95	2.0	1.8	2.0	1.8	1.65	1.55	0.6
9 MPV	2.0	1.75	1.6	1.45	1.95	1.65	1.85	1.65	1.85	1.75	1.95	1.65	1.5	1.5	0.75
10 MPV	1.65	1.5	1.35	1.2	1.55	1.3	1.65	1.35	1.65	1.5	1.55	1.4	1.1	1.2	0.75

N.B. Specific protection S/P > 1.0 is considered positive.

WPV: Weeks Post Vaccination

MPV: Months Post Vaccination.

Group (1): Calves vaccinated with FMDV trivalent vaccine.

Group (2): Calves vaccinated with FMD trivalent vaccine and posted with BEFV vaccine 4 WPV.

Group (3): Calves vaccinated with FMD trivalent vaccine and SPV vaccine.

Group (4): Calves vaccinated with FMD trivalent vaccine, BEFV vaccine and SPV vaccine and posted with BEFV 4 WPV.

Group (5): Calves vaccinated only with SPV vaccine.

Group (6): Calves vaccinated only with BEFV vaccine and posted with BEFV vaccine 4 WPV.

Group (7): Control unvaccinated calves.

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الملخص العربي

تأثير لقاحى حمى الثلاث أيام، لقاح جدري الأغنام على الحالة المناعية للعجول المحصنة بلقاح الحمى القلاعية الثلاثى الزيتى

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فى هذه الدراسة تم تحصين عجول بلقاحات حمى الثلاث أيام المثبط، جدري الأغنام المستضعف والحمى القلاعية الثلاثى المثبط كلاً على حدة، أو فى نفس الوقت حيث تم تقسيم واحد وعشرون عجل الى سبع مجموعات (كل مجموعة ثلاث عجول). الاستجابة المناعية الخلوية قد درست بواسطة اختبار ال MTT معتمداً خلايا الليمفوسايت فى عينات الدم وعند الحقن المتزامن لفيروس حمى الثلاثة أيام والحمى القلاعية لم تتأثر المناعة الخلوية ولكن عند حقن فيروس لقاح جدري الأغنام أو الحمى القلاعية مع جدري الأغنام وحدهما تكون المناعة الخلوية فى مستوى مرتفع ومبكرة فى الظهور. من جهة أخرى لم توجد اختلافات فارقة فى مستوى الاجسام المناعية بواسطة كلاً من اختبار الاليزا والمصل المتعادل بين مجموعات الحيوانات المحصنة بلقاح الحمى القلاعية وحمى الثلاث ايام، بينما وجدت زيادة معنوية فى مستويات الاجسام المناعية سجلت فى حالة التحصين بلقاحات جدري الأغنام وحمى الثلاث أيام مع الحمى القلاعية فى نفس الوقت. على ذلك يمكن القول ان التحصين التزامنى للعجول بلقاحات جدري الأغنام وحمى الثلاث ايام مع الحمى القلاعية الثلاثى يعتبر مفيداً عملياً.