Evaluation of an inactivated BEF virus vaccine adjuvant on montanide ISA 206 in cattle subjected for one and two doses immunization programs.

El-Bagoury, G.F.[1]; El-Habbaa, A.S.[1]; Amal, A.M. [2]; and Nermeen, G.S.[2]

[1] *Department of Virology, Faculty of Veterinary Medicine, Benha University.*
[2]*Central Laboratory for Evaluation of Veterinary Biologics, Abbassia, Cairo.*

ABSTRACT

Bovine ephemeral fever (BEF) is an infectious, arthropod-born viral disease has economic importance in cattle and buffaloes. Vaccination is widely used to control and prevent BEF disease. This study aimed to prepare an inactivated BEF vaccine using Montanide ISA 206 (water-in-oil-in-water) adjuvant that was applied in two different protocols (one dose and two doses one month apart). The prepared vaccine was sterile and safe. It was found that a single dose of the vaccine induced protective neutralizing serum antibody titer from 2nd week post vaccination (PV), reached highest titer at 10th week PV and persisted in this protective titer until 34 weeks PV using SNT and confirmed using ELISA, however boostering of animals 4 weeks post preliminary vaccination increased the titer of the protective neutralizing antibodies and its time duration to 44 weeks PV. Thus, in order to provide immunity that will last the entire season it is recommended that the vaccine should be administered short time before the onset of BEF season (summer season).

KEYWORDS: BEF, Vaccine, Montanide ISA 206, SNT, ELISA.

(http://www.bvmj.bu.edu.eg) **(BVMJ-31(2): 241-247, 2016)**

1. INTRODUCTION

Bovine ephemeral fever (BEF) is an infectious, arthropod-born viral disease of cattle and water-buffaloes characterized by sudden onset of fever, depression, difficulty swallowing, serous ocular and nasal discharge, dyspnea, stiffness and lameness (Nandi and Negi, 1999). Various complications could occur as pneumonia, subcutaneous emphysema, mastitis, hind-quarter paralysis and bull infertility for up to six months (Radostits et al., 2000). Financial effects ascribed to BEF included intense drop in milk production, abortion, expenses of care of infected animals and immunization of healthy flocks and disturbance of routine stock handling, care and marketing (Coetzer et al., 1994). The causative BEF virus was characterized in genus Ephemerovirus in the family Rhabdoviridae and has bullet or cone-shaped morphology, single stranded negative-sense RNA genome (14,900 nucleotides) and five structural proteins involving a nucleoprotein (N), a polymerase-associated protein (P), a matrix protein (M), a large RNA-dependent RNA polymerase (L) and a glycoprotein (G) spreading over the viral envelope against which neutralizing antibodies are directed (Walker et al., 1991). The virus is transmitted by hematophagous insects that it has been isolated from mosquitoes and Culicoides biting midges (Kirland, 1995; Mellor, 1996). All perceived episodes of BEF infection have regular appearance which principally happened in late summer and autumn and happen over an expansive range of tropical, subtropical and temperate countries in Africa, Asia, Middle East and Australia (St George, 1988). In Egypt, several outbreaks of BEF have been happened in summer of rehashed years (Al-Gaabary et al., 2005 ; Davies et al., 1993; El-Bagoury et al., 2014; Hassan, 2000; Kawther and Wahid, 2011; Zaghawa et al., 2002). Vaccination is widely used to control and prevent BEF disease. Local live and inactivated BEF vaccines were prepared and successfully used to give good protection rates (Daoud et al., 2001a; Daoud et al., 2001b). However, inactivated BEF vaccine showed short duration of protection among vaccinated animals and repeated occurrence of BEF among cattle and buffaloes at different Egyptian provinces was registered (Hassan, 2000). Recently, inactivated vaccines have used water-in-oil-in-water adjuvant that shown to elicit a stronger and longer lasting neutralizing antibody response, depend on booster programs (Aziz-Boaron et al., 2013).

We have organized our work to prepare an inactivated BEF vaccine using Montanide ISA 206 (water-in-oil-in-water) as adjuvant then to appraise the duration of immune response in animals taking the vaccine by two particular traditions (one and two doses).

2. MATERIAL AND METHODS

2.1. Bovine Ephemeral Fever (BEF) virus strain:

It was isolated from Toukh Tambasha; Monofia Governorate, Egypt during the outbreak that occurred in summer 2000 (Soad et al., 2001). This isolate designed as Abbassia virus strain (BEF/AVS/2000), was supplied by Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt.

2.2. Baby Hamster Kidney cell line (BHK21 clone 13):

It was supplied by the Animal Research Institute, Pirbright, UK. It was propagated at FMD Department, Veterinary Serum and Vaccine Research, Institute, Abbasia, Cairo, Egypt by using of minimum Essential Medium (MEM) with Eagle's salts and with 10 % new born calf serum according to (Macpherson and Stocher, 1962). These cells were used for virus propagation and titration and also for SNT

2.3. Experimental Calves:

Fourteen male calves (local breed) aged between 9-10 months old about 300 kg body weights were used for evaluation of the prepared inactivated BEF vaccine with Montanide ISA206 adjuvant.

2.4. Vaccine formulation:

BEF virus was inoculated onto a monolayer BHK-21 cell line that harvested at over 70% cytopathic effect after 30–35 h, and then it was purified by centrifugation at 3000 rpm for 20 minutes to remove cell debris (Killington et al., 1996). Titer of the tissue culture adapted virus was estimated on BHK21 cell line (Reed and Muench, 1938) and its antigenicity was titrated using Complement Fixation Test (CFT), ((Traub and Manso, 1944). The seed BEF virus had a titer of 10^8TCID50/ml and 64 using infectivity titration and CFT, respectively. It was inactivated by Binary ethylenimine (BEI) and the vaccine formulation was carried out where the oil phase consisted of Montanide ISA 206, mixed as equal parts of an aqueous and oil phase weight/ weight, and mixed to make water-in-oil-in-water suspension (Barnett et al., 1998).

2.5. Quality control of the prepared vaccine:

Sterility and safety was evaluated for the prepared inactivated BEF vaccine according to (Code of Federal Regulation of USA, 1986). Sterility test was applied to confirm that the prepared vaccine was free from bacterial and fungal contamination using nutrient agar, thioglycolate broth (for bacterial detection) and Sabouraud's dextrose agar (for fungal detection). Safety of the inactivated BEF virus before addition of adjuvant was applied on tissue culture and the safety of the prepared vaccine after adjuvant was applied by injection of 20 ml (10X dose) of the vaccine in calves by subcutaneous (S/C) route.

2.6. Experimental design:

Calves were used for evaluation of the prepared inactivated BEF vaccine with Montanide ISA206 adjuvant as follow: Group (1): Five calves vaccinated with 2ml/animal (S/C) of the prepared oil vaccine as one dose. Group (2): Five calves vaccinated with 2ml/animal (S/C) of the prepared oil vaccine and boostered similarly after one month. Group (3): Two calves were kept as control without vaccination. Group (4): Two calves were used in safety test of the prepared vaccine. Sera from animals in groups (1), (2) and (3) were collected for 48 weeks and used to measure the variance in efficacy and duration of immunity in vaccinated calves using SNT and ELISA test.

2.7. Serum neutralization test (SNT):

SNT was performed using the micro-technique to detect neutralizing antibodies against BEF virus. The antibody titer was estimated as the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 $TCID_{50}$ of BEF virus (Mellor, 2001).

2.8. Enzyme Linked Immunosorbent Assay (ELISA):

BEF antigen was prepared from infected BHK - 21 cells and concentrated by PEG (6000) according to (Wagner et al., 1969), then used in ELISA to estimate the specific antibodies of BEF virus (Zakrzewski et al., 1992).

3. RESULTS

3.1. Sterility and safety of the vaccine:

The prepared vaccine was free from aerobic and anaerobic bacteria and fungi. It was also safe and gave satisfactory results indicated by absence of cytopathic effect on tissue culture, and absence of local and systemic reactions on inoculated calves with no rise in body temperature.

3.2. Humeral immune response:

Evaluation of the humeral immune response in vaccinated calf groups using SNT showed that protective neutralizing serum antibody titer (1.2) started from 2nd week PV, reached highest titer at 10th week PV and persisted in this protective titer until 34 weeks PV for group (1) of calves vaccinated with one dose of the prepared vaccine. For group (2) of calves vaccinated with two doses of the prepared vaccine one-week interval, protective neutralizing serum antibody titer started from 2nd week PV, reached the highest level at 12th week PV and persisted in the protective level until 44 weeks PV. These results were shown in table (1) and figure (1) in comparison to that of the calves kept as control non-vaccinated Group (3).

Table (1): Neutralizing antibody titers in sera from calves vaccinated with the prepared inactivated BEF vaccine with Montanide oil ISA206 adjuvant) using SNT:

Weeks post vaccination	Mean log_{10}serum neutralizing antibody titers		
	Vaccinated calf groups		Control
	One dose	Two doses	Non-vaccinated
1	*0.75	0.75	0
2	1.38	1.35	0
3	1.8	1.65	0
4	1.98	1.98	0
6	2.16	2.22	0
8	2.43	2.46	0
10	2.55	2.58	0
12	2.46	2.7	0
14	2.28	2.58	0
16	2.22	2.46	0
18	2.01	2.34	0
20	1.83	2.22	0
22	1.62	2.07	0
24	1.53	1.89	0
26	1.44	1.83	0
28	1.38	1.74	0
30	1.29	1.62	0
32	1.23	1.56	0
34	1.2	1.5	0
38	1.02	1.41	0
40	0.9	1.29	0
44	0.75	1.23	0
48	0.42	0.99	0

Protective serum neutralizing antibody titer=1.2

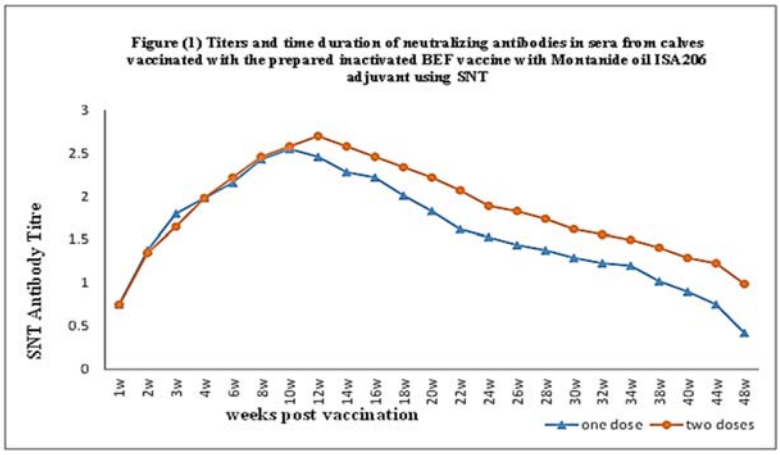
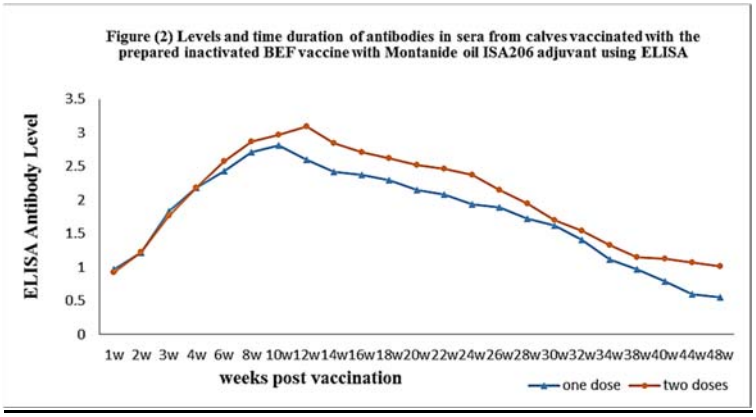


Table (2): Level of antibody in sera from calves vaccinated with the prepared inactivated BEF vaccine with Montanide oil ISA206 adjuvant) using ELISA:

Weeks post vaccination	Mean optical densities of examined sera		
	Vaccinated calf groups		Control non-vaccinated
	One dose	Two doses	
1	0.965	0.9194	0.220
2	1.218	1.2256	0.214
3	1.8388	1.7614	0.204
4	2.1852	2.1826	0.215
6	2.4266	2.5752	0.198
8	2.7102	2.8658	0.213
10	2.8152	2.9676	0.224
12	2.595	3.0928	0.223
14	2.4168	2.8436	0.196
16	2.3716	2.713	0.198
18	2.294	2.6256	0.213
20	2.149	2.5186	0.190
22	2.0824	2.4582	0.210
24	1.9356	2.3678	0.193
26	1.888	2.1476	0.196
28	1.7254	1.9446	0.204
30	1.6222	1.6974	0.190
32	1.404	1.545	0.202
34	1.1104	1.3288	0.197
38	0.9728	1.1504	0.203
40	0.7936	1.1222	0.193
44	0.596	1.067	0.194
48	0.5584	1.016	0.198

Positive results=optical density more than one.



Evaluation of the humeral immune response in vaccinated calf groups using ELISA showed that serum antibody level started to increase from the 2nd week PV, reached highest level at 10th week PV and persisted in this satisfactory level until 34 weeks PV for group (1) of calves. For group (2), calf's serum antibody level started to increase from 2nd week PV, reached the highest level at 12th week PV and persisted in the satisfactory level until 48 weeks PV. These results were shown in table (2) and figure (2) in comparison to that of the calves kept as control non-vaccinated Group (3).

4. DISSCUSSION

In Egypt, BEF emerged as a major viral disease of cattle and many outbreaks have been occurred. The economic impact of BEF was not estimated until the epidemic of 2000-2001 (Zaghawa et al., 2002) and the frequent recurrence since then which coasted a hundred million of Egyptian pounds. In order to preclude production losses caused by BEF, vaccination of cattle particularly dairy and feedlot herds and valuable breeding stock was carried out to ensure a high level of immunity in the offending

season when vector arthropods are likely to be most abundant. Advancement in the decade of ready-to-formulate oil adjuvant has added a new dimension to vaccine formulation. Oil adjuvant (water-in-oil-in-water) contains all components necessary to produce complex, low viscosity, stable emulsion which safely stimulates rapid and protective immune response, in addition this adjuvant has been found to stimulate longer immunity compared to aluminum salts adjuvant (Hsieh et al., 2006).

In our study an inactivated BEF vaccine was prepared using Montanide ISA206 (water-in-oil-in-water) as an adjuvant. Sterility of the prepared vaccine was proved being free from bacterial and fungal contamination (Code of Federal Regulation of USA, 1986). The prepared vaccine proved to be safe in both tissue culture and calves. In tissue culture, inoculated BHK21 cell line showed no cytopathic effect, and the safety in calves gave also no rise in body temperature and no clinical abnormalities (Henderson, 1970).

Comparative evaluation of the humeral immune response in vaccinated calves showed that protective neutralizing serum antibody titer (1.2) started from 2nd week post vaccination (PV), reached highest titer at 10th week PV and persisted in this protective titer until 34 weeks PV for group (1) of calves vaccinated with one dose of the prepared vaccine. The previous results were assessed in comparison to that of the control non-vaccinated calves using SNT that were confirmed using ELISA. These results came in agreement with that of Barnett et al. (1996); Cox et al. (2003); Hunter (1996); Patil et al. (2002) who suggested that vaccines adjuvanted with Montanide ISA 206 can promote longer lasting immunity than conventional vaccines adjuvanted with Alhydragel in ruminant persisted for at least 6 months' post vaccination.

In the other side, protective neutralizing serum antibody titer started from 2nd week PV, reached the highest level at 12th week PV and persisted in the protective level until 44 weeks PV for group (2) of calves vaccinated with two doses of the prepared vaccine one-week interval. The previous results were assessed in comparison to that of the control non-vaccinated calves using SNT that were confirmed using ELISA. These results came in agreement with that of Theodoridis et al. (1973) who used emulsions of BEF virus of both low and high passage in Freund's incomplete mineral oil adjuvant and noticed that this vaccine could induced high neutralizing antibodies in vaccinated cattle which could be detectable for at least a year. These results came in agreement also with that of Cameron et al. (1987) who reported that the booster BEF oil emulsion vaccine or double injection give very good antibody response and high titer than single injection.

This study reflected that single dose of inactivated BEF vaccine on Montanide oil ISA 206 (water-in-oil-in-water) adjuvant gave a long immunity for not less than 8 months PV but when these animals were bostered by a second dose 4 weeks post preliminary vaccination, the titer of the neutralizing antibodies and its time duration were increased to reach 44 weeks PV. Thus, in order to provide immunity that will last the entire season it is recommended that the vaccine should be administered a short time before the onset of BEF season (summer season).

5. REFERENCES

Al-Gaabary, M.H., Osman, S.A., Foad, F., 2005 Comparative clinical and epidemiological studies on bovine ephemeral fever in Sakha farm, KafrEl-Sheikh, Egypt. Assiut Veterinary Medical Journal 51, 76-85.

Aziz-Boaron, O., Leibovitz, K., Gelman, B., Kedmi, M., Klement, E., 2013. Safety, immunogenicity and duration of immunity elicited by an inactivated bovine ephemeral fever vaccine. PLoS One 8, e82217.

Barnett, P.V., Pullen, L., Warder, P., Stathen, R., 1998. International bank for foot and mouth disease vaccine (preliminary studies on emergency foot and mouth disease vaccines formulated with montanide IMS -Immunosol-, a new concept in oil adjuvancy. European commission for the control of FMD. Aldershot, United Kingdom.

Barnett, P.V., Pullen, L., Williams, L., Doel, T.R., 1996. International bank for foot-and-mouth disease vaccine: Assessment of Montanide ISA 25 and ISA 206, two commercially available oil adjuvants. Vaccine 14, 1187-1198.

Cameron, C.M., Barnard, B.J.H., Erasmus, E., Botha, W.J.S., 1987. Antibody response in cattle to oil emulsion rabies and ephemeral fever vaccines. Onderstepoort journal of veterinary research 54, 157-158.

Code of Federal Regulation of USA, 1986. Published by the office of the federal register national archives and Record administration. Animal and animal products.

Coetzer, J.A.W., Thomson, R.G., Tustin, R.C., Kriek, N.P.J., 1994. In infectious diseases of

livestock. Oxford University Press, Cape Town Oxford, New York.

Cox, S.J., Aggarwal, N., Statham, R.J., Barnett, P.V., 2003. Longevity of antibody and cytokine responses following vaccination with high potency emergency FMD vaccines. Vaccine 21, 1336-1347.

Daoud, A.M., Azab, A., Khodeir, M.H., El-Gallad, S.B., Amany, A.S., 2001a. Antigenic relationship between bovine ephemeral fever (BEF) and rabies viruses. Beni-Suef Veterinary Medical Journal 11, 229-236.

Daoud, A.M., Saber, M.S., Taha, M.M., Azab, A., Soad, M.S., 2001b. Preparation of attenuated bovine ephemeral fever (BEF) vaccine. Beni-Suef Veterinary Medical Journal 11, 627-634.

Davies, F.G., Moussa, A., Barsoum, G., 1993. The 1990-1991 epidemic of ephemeral fever in Egypt and the potential for spread to the Mediterranean region.Bovine ephemeral fever and related rhabdoviruses, In: Proceedings of the 1st International Symposium held in Beijing, Beijing, pp. 54-56.

El-Bagoury, G.F., El-Nahas, E.M., El-Habbaa, A.S., 2014. Serological and molecular identification of Bovine Ephemeral Fever virus isolates from cattle and buffaloes in Qaluobia province, Egypt 2014. Egyptian Journal of Virology 11, 176-182.

Hassan, H.Y., 2000. An outbreak of bovine ephemeral fever in Egypt during 2000. II. Clinico-chemical and hematological alternations. Assiut Univ. Egypt., Fac. Vet. Med., pp. 354-356.

Henderson, W.M., 1970. Foot and mouth disease; a definite ion of the problem and view on its solution. Br.Vet. J. 126, 115-120.

Hsieh, Y.C., Wang, S.Y., Lee, Y.F., Chen, S.H., Mak, P.O.T., Chu, C.Y., 2006. DNA sequence analysis of glycoprotein G gene of bovine ephemeral fever virus and development of a double oil emulsion vaccine against bovine ephemeral fever. J. Vet. Med. Sci. 68, 543-548.

Hunter, P., 1996. The performance of Southern African Territories serotypes of foot and mouth disease antigen in oil adjuvanted vaccine. Rev.Sci.Tech., 15, 913-922.

Kawther, S.Z., Wahid, M.A., 2011. Investigation of Bovine Ephemeral Fever Virus in Egyptian cows and buffaloes with emphasis on isolation and identification of a field strain. Global Veterinaria 6, 447- 425.

Killington, R.A., Stokes, A., Hierolzer, 1996. Virology methods manual. Chapter 4: 72-89.

Kirland, P.D., 1995. The epidemiology of bovine ephemeral fever in Southwestern Australia: evidence for a mosquito vector. In 1st International Symposium (Beijing) on bovine ephemeral fever and related arboviruses. ACIAR Proceedings, Canberra 44, 33-37.

Macpherson, M., Stocher, B., 1962. Polyma transformation hamster cell clones, an investigation of genetic factors affecting cell competence. Virology 16, 147-151.

Mellor, P.S., 1996. Culicoides: vectors, climate change and disease risk. Vet. Bull. 66, 301-306.

Mellor, P.S., 2001. Bovine ephemeral fever. In encyclopedia of arthropod-transmitted infections of man and domesticated animals. Edited by Service, M.W.; Ashford, R.W.; Calidher, C.H.; Eldridge, B.F.; Jones, T.W. and Wyatt, G. . CABI Publishing.

Nandi, S., Negi, B.S., 1999. Bovine ephemeral fever: a review. Comp Immunol Microbiol Infect Dis 22, 81-91.

Patil, P.K., Bayry, J., Ramakrishna, C., Hugar, B., Misra, L.D., Natarajan, C., 2002. Immune response of goats against FMD quadrivalent vaccine: comparison of double oil emulsion and aluminum hydroxide gel vaccine in eliciting immunity. Vaccine
20, 2781-2789.

Radostits, O.M., Gay, C.C., Blood, D.C., Hinchcliff, K.W., 2000. Veterinary Medicine, 9th ed. W.B. Saunders Co. Ltd., London, New York, Philadelphia, Sydney. Printed in China.

Reed, L.J., Muench, H., 1938. A simple method for estimating fifty percent (50%) end points. Amer. J. Hyg. 27, 493-497.

Soad, M.S., Taha, M.M., Samir, S.S., Daoud, A.M., 2001. Isolation and identification of bovine ephemeral fever virus in Egypt. Beni-Suef J. Vet. Med. 11, 609-617.

St George, T.D., 1988. Bovine ephemeral fever: A review. Trop. Anim. Health Prod. 20, 194-202.

Theodoridis, A., Boshoff, S.E.T., Botha, M.J., 1973. studies on the development of a vaccine against bovine ephemeral fever. Onderstepoort J. Vet. Res. 40, 77-82.

Traub, E., Manso, I.R., 1944. Uber die herstellung complement bindender merrsch-weinehon sera fur die typendiagnose bie Maul und Klauenseuche. Zbi. Bakt. Lorig. 151, 380-381.

Wagner, G.G., Card, J.L., Cowan, K.M., 1969. Plum Island Animal Disease Laboratory, Animal Disease and Parasite Research

Division Agricultural Research Service. Department of Agricultural, Greanport, New York, USA.

Walker, P.J., Byrne, K.A., Cybinski, D.H., Doolan, D.L., Wang, Y., 1991. Proteins of bovine ephemeral fever virus. J. Gen. Virol. 72 67–74.

Zaghawa, A., Akela, A.M., Khadr, A.M., Hassan, H.Y., 2002. An outbreak of bovine ephemeral fever in Egypt during 2000. I. Clinical and epidemiological investigations. Assiut Univ, Egypt, Sci. Cong. 2000, Fac. Vet. Med., pp. 346-353.

Zakrzewski, H., Cybinski, D.H., Walker, P.J., 1992. A blocking ELISA for the detection of specific antibodies to bovine ephemeral fever virus. J. Immunol. Methods 151, 289-297.